CLINICAL PHARMACOKINETICS OF TACROLIMUS IN ASIAN LIVER TRANSPLANT PATIENTS

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PUBLICATIONS

International Refereed Publication

1) W.J. Sam, M. Aw, S.H. Quak, S.M. Lim, B.G. Charles, S.Y. Chan & P.C. Ho. Population pharmacokinetics of tacrolimus in Asian paediatric liver transplant patients. *British Journal of Clinical Pharmacology* 2000; **50**: 531-541.

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LIST OF ABBREVIATIONS

Abbreviation	Full name
AGE	Age
AIC	Akaike's information criterion
ALB	Albumin
ALT	Alanine amino transferase
APC	Antigen-presenting cells
APH	Alkaline phosphatase
AST	Aspartate amino transferase
AUC	Area under curve
BSA	Body surface area
CL	Clearance
CL/F	Apparent clearance
C_{max}	Maximum drug concentration
C_{min}	Minimum drug concentration
CREA	Creatinine
CV	Coefficient of variation
CYP	Cytochrome
F	Bioavailability
FO	First-order
FOCE	First-order conditional estimation
GAM	Generalized additive modeling
GEN	Gender

GGT	Gamma glutamyl transferase
HCT	Haematocrit
HPLC/MS/MS	High-performance liquid chromatographic/mass spectrophotometric/mass spectrophotometric
HLA	Human leukocyte antigen
HT	Height
IFN	Interferon
IL	Interleukin
IV	Intravenous
LDH	Lactate dehydrogenase
LOQ	Limit of quantification
ME	Mean prediction error
MHC	Major histocompatibility complex
MFO	Mixed function oxidase
MDR-1	Multidrug-resistance 1
MRM	Multiple-reaction monitoring
MS	Mass spectrometric
NF-AT	Nuclear factor of activated T cells
NONMEM	Nonlinear mixed effects model
OBJF	Objective function
OKT3	Muromonab-CD3
P-glycoprotein	Permeability-glycoprotein
PD	Pharmacodynamics
PK	Pharmacokinetics

POD	Post-operative days
PROT	Total protein
RMSE	Root mean square error
RSS	Residual sum of squares
SNP	Single nucleotide polymorphism
SD	Standard deviation
t_{abs}	Absorption half-life
$t_{½}$	Elimination half-life
t_{max}	Time to maximum blood concentration
Tac	Tacrolimus
TBM	Tree-based modeling
TDM	Therapeutic drug monitoring
TLI	Total lymphoid irradiation
TNF	Tumour necrosis factor
TOTBIL	Total bilirubin
UREA	Urea
V	Volume of distribution
V/F	Apparent volume of distribution
V_{SS}	Volume of distribution at steady state
WT	Weight

SUMMARY

The general aim of the thesis is to investigate the population pharmacokinetics (PK) of tacrolimus (Tac) (FK506) in the local Asian liver transplant recipients so as to identify possible relationships between clinical covariates and population parameter estimates. This can be achieved by (A) determining the population PK of Tac in the local Asian paediatric liver transplant recipients, which represents a special subpopulation with special PK characteristics, and (B) determining the population PK of Tac in the local Asian adult and paediatric liver transplant recipients.

In the first study, the population pharmacokinetics (PK) of intravenous and oral Tac was determined in 20 Asian paediatric patients, aged 1-14 years, after liver transplantation. Population modeling using the nonlinear mixed effects model (NONMEM) program was performed on the population data set, assuming a one-compartment model with first-order absorption (k_a fixed to 4.5 hr^{-1}) and elimination.

The final optimal population models identified the following relationships: CL (L/hr) = 1.46 • [1 + 0.339 • (AGE (yr) – 2.25)]; V (L) = 39.1 • [1 + 4.57 • (BSA (m^2) – 0.49)]; F = 0.197 • [1 + 0.0887 • (WT (kg) – 11.4)] and F = 0.197 • [1 + 0.0887 • (WT (kg) – 11.4)] • [1.61], if the total bilirubin ≥ 200 μmol/L. The mean population estimates of CL, V and F were 1.46 L/hr, 39.1 L and 0.197, respectively. The interpatient variabilities (CV %) in CL, V and F were 33.5 %, 33.0 % and 24.1 %, respectively.

For the second study, the population PK of oral Tac was determined in 31 Asian adult and paediatric patients, aged 1-67 years, after liver transplantation. A one-compartment PK model with first-order absorption and elimination was used to describe the disposition of Tac in these patients.

The final optimal population model related CL/F to body weight, the level of serum creatinine and the level of alkaline phosphatase, and produced the following relationship: CL/F (L/hr) = 14.1 + 0.237*[WT (kg)-55] – 0.0801*[CREA (μmol/L)-60] and CL/F (L/hr) = 14.1 + 0.237*[WT (kg)-55] – 2.93 – 0.0801*[CREA (μmol/L)-60], if the alkaline phosphatase is ≥ 200 U/L. The final optimal population model related V/F to body height and the level of haematocrit of the patient, and produced the following relationship: V/F (L) = 217 – 7.83*[HCT (L/L)-31.1] + 179*[HT (m)-1.61].

The mean population estimates of CL/F and V/F in the Asian adult and paediatric liver transplant patients were 14.1 L/hr and 217 L, respectively. Reasonably large interindividual variabilities of 65.7 % and 63.8 % were estimated for CL/F and V/F, respectively.

The population models have identified significant relationships in Asian liver transplant patients between the PK of Tac and arthropometric characteristics of the patients, as well as the clinical conditions of the patients. Using these models, in conjunction with Bayesian forecasting, a truly individualized immunosuppressive therapy can be developed and applied.

INTRODUCTION

1.1. Transplantation Immunology

1.1.1. Clinical transplantation

Transplantation is the act of transferring cells, organs or tissues from one site or individual to another. It provides effective treatment for various end-stage organ diseases of the heart, lung, cornea, liver, kidney and bone marrow

As the majority of the transplants is performed between genetically different individuals of the same species (allografts), the immunological response of the recipient to the antigens from the donor graft, must be considered. The result of this immune response, if left unchecked, will lead to the rejection of the transplanted tissue and both cellular (or lymphocyte-mediated) and humoral (antibody-mediated) mechanisms are involved.

1.1.2. Clinical characteristics of allograft rejection

Rejection can be defined as graft damage arising from the response of the recipient's immune system to the transplanted organ. It involves cell- and antibody-mediated organ injury occurring as the result of recognition of allograft as nonself (Arakelov and Lakkis, 2000). This process is caterorised into three major types: hyperacute, acute, and chronic.

Hyperacute rejection occurs when an abrupt loss of allograft function occurs within minutes to hours after circulation is established in the allograft. This process is mediated by preexisting antibodies to allogeneic antigens on the vascular endothelial cells within the donor organ (Hanto *et al.*, 1987). These antibodies fix complement thereby promoting intravascular thrombosis and leading to rapid occlusion of graft vasculature and rapid rejection of the graft (Hammond *et al.*, 1993). Donor recipient human leukocyte antigen (HLA) and ABO blood-group cross-matching are used to prevent hyperacute rejection (Abbas *et al.*, 1994). There is currently no immunosuppressive therapy effective in managing hyperacute rejection. Clinical symptomatology associated with hyperacute rejection varies depending on allograft type, but typically reflects intensive organ failure.

Acute rejection may occur within days of transplantation in the untreated recipient or may appear suddenly months or even years later, when immunosuppression has been employed and terminated. It is a combined process in which both cellular and humoral tissue injuries play a part (Oluwole *et al.*, 1989). However, cell-mediated immunity mediated by T cells is the primary cause of acute rejection in any one patient. It is characterized by necrosis of parenchymal cells within the donor organ. Acute rejection can be initiated when graft injury produces an up-regulation of adhesion molecules on endothelial cell lining blood vessels in the graft (Nair and Morris, 1995). Host T-cell receptors bind to these adhesion molecules increasing their transit time through graft vessels and promoting their migration into allograft tissue. Subsequently two varieties of cells – T lymphocytes and antigen-presenting cells (APCs) – are recruited (Billingham *et*

al., 1956). The professional APCs are critical to the immune response. They include dendritic cells and macrophages that bind antigen and present it to T cells in the form of short peptides bound to the major histocompatibility complex (MHC) of APCs (Rammensee *et al.*, 1993; Germain, 1994). MHC is one set of the many histocompatibility molecules that has a predominant influence on tissue compatibility and is by far the most polymorphic (Klein *et al.*, 1993). The gene products of the MHC molecules in humans are called human leukocyte antigen (HLA). There are two main types of MHC molecules that are important for allograft transplantation: class I and II. The HLA class I molecules are present on all nucleated cell surface to display antigenic peptides to the cytotoxic cells (Harris and Gill, 1986). In contrast class II molecules are found almost exclusively on cells associated with the immune system: the professional APC found in lymphoid tissues and activated T cells (Daor *et al.*, 1984).

Macrophage and polymorphonuclear leukocyte activity dominates the early phase of tissue injury. Macrophages produce cytotoxic mediators such as interleukin-1 (IL-1) and tumour necrosis factor (TNF) upon recognition of pathogens or foreign antigens. These cytokines function to increase recruitment of of T-cells to the graft site. The activation of T-cells is the primary factor associated with acute rejection (Gowans *et al.*, 1962). Activated T-cells also secrete inflammatory cytokines, which interfere in microvascular processes to an extent that vascular insufficiency contributes to endothelial cell death. Clinical signs which may be associated with acute rejection include general malaise, fatigue, myalgia, low-grade fever and pain or tenderness at the graft site (except in heart transplant as surgical denervation processes cannot be completely reversed).

Other symptoms may also be observed, such as hypertension, weight gain, decreased urine output and increased serum creatinine in acute kidney rejection.

Chronic rejection, or late graft failure, is an irreversible gradual deterioration of graft function that occurs in many allografts months to years after transplantation (Häyry *et al.*, 1993). It is characterized by intimal thickening and fibrosis leading to luminal occlusion of the graft vasculature (Abbas *et al.*, 1994). For example, cardiac allograft vasculopathy has been detected angiographically in 44% of heart transplant recipients at 3 years (Uretsky *et al.*, 1987). This form of rejection involves a variety of immune-system components: T cells, cytokines, macrophages, and adhesion molecules (Azuma and Tilney, 1994). Both immunologic and nonimmunologic factors appear to be involved in the ultimate impairment of organ functions. Acute rejection episodes, inadequately treated acute rejection, insufficient long-term immunosuppression therapy, preservation injury, lipid abnormalities, and infection have all been associated with chronic rejection (Ventura *et al.*, 1995).

The immunology of allograft rejection is not yet fully understood. However, the increasing insight into the complexities of these host mechanisms holds promise for further improvement in knowledge, their attenuation by both chemical and biological agents and eventual success in the production of transplant tolerance, which is the long-term acceptance of the allograft by the recipient.

1.1.3. Prevention or reduction of rejection

Since the vast majority of transplants come from incompatible unrelated donors, nonpharmacologic and pharmacologic means of immunosuppression can be given to control transplant immunity so that rejection can be prevented or controlled and that the recipient develops a long-term acceptance (or tolerance) of the transplant.

Nonpharmacologic immunosuppression

Total lymphoid irradiation (TLI) is considered an alternative immunosuppressive therapy for highly sensitised patients who have received prior organ transplantation (Slavin *et al.*, 1980). By targeting x-rays to lymphoid tissues, using small fractionated doses to minimize side effects, and discontinuing therapy with the appearance of adverse effects, TLI has been used successfully in renal transplant patients in combination with low doses of immunosuppressive drugs (Saper *et al.*, 1988).

In some cases, prior intravenous exposure to donor antigens (especially by blood transfusions) can cause prolonged or indefinite graft survival, even though one might expect hyperacute graft rejection to occur. This phenomenon is called “active enhancement of graft survival.” Active enhancement of graft survival has been employed clinically using donor specific transfusions (Newton and Anderson, 1979).

Pharmacologic immunosuppression

Pharmacologic agents currently provide the primary means of immunosuppression after transplantation. Over the past 40 years, compounds that block distinct aspects of the immune response have been developed. Immunosuppressive agents can be classified according to their mechanisms of action as shown in Table 1.

Mechanism of action	Example
Lymphokine synthesis inhibitors dependent on the immunophilin-calcineurin complex	Cyclosporine, Tacrolimus (Tac)
DNA (nucleotide) synthesis inhibitors	
a. purine antagonists	Azathioprine, mycophenolate mofetil
b. pyrimidine antagonists	Brequinar sodium, leflunomide
c. folate antagonists	Methotrexate
d. alkylators	Cyclophosphamide
Growth factor / cytokine action modulators (inhibitors)	Rapamycin, leflunomide
Multiple actions / unknown mechanisms of action	Prednisone, 15-deoxyspergualin
Anti-lymphocyte / endothelial cell biological agents	Polyclonal anti-lymphocyte globulin, muromonab-CD3 (OKT3), anti-CD4, anti-T10, anti-CD52, anti-ICAM-1
Pharmacologic and biologic agents for positive and negative selection of bone marrow	Cyclophosphamide analogs, anti-CD34 monoclonal antibodies

Table 1. Classification and mechanisms of action of drug and biological agents currently used in transplantation.

Drug combinations hold the greatest promise for managing transplant-related immunosuppression. As discussed previously, the allograft rejection process involves both T and B cells, multiple cytokines, and inflammatory mediators. Selective drug combinations that prevent compensatory immune mechanisms from avoiding suppression

and that take advantage of proven synergism between agents should provide the most effective therapy. Drug combinations also allow the use of minimal effective doses of immunosuppressive agents so that drug toxicity is diminished.

The development of immunosuppressive regimens since the mid-1960s has resulted in the current use of "triple therapy" or "quadruple therapy" as the standard regimen in most transplant centers. Triple therapy, which is low dose cyclosporine or Tac, azathioprine, and steroids, takes advantage of the immunosuppressive effects of cyclosporine or Tac while minimizing their adverse effects. Quadruple therapy adds anti-lymphocyte globulin or OKT3 to triple therapy with a delay in the start of cyclosporine until adequate renal function is established (Deierhoi *et al.*, 1987).

Optimal immunosuppressive therapy entails a careful management of the patient, which includes proper dosing strategies, measurement of the drug levels in the blood, monitoring of graft function using biopsy histology and evaluation of potential side effects of the drug.

1.2. Tacrolimus

1.2.1. Discovery and development

In 1982, workers from Fujisawa Pharmaceuticals (Ibaraki, Japan) started to test a wide range of fermented broths from *Streptomyces* for specific inhibitory effects on

mixed lymphocytes cultures. In 1984, as a result of this screening, strain no. 9993 was found to produce a potent immunosuppressant designated by the code number FK506, later named Tac (Prograf®). The strain has been designated as *Streptomyces tsukubaensis*, referring to the origin of the soil (Kino *et al.*, 1987). In 1994, Tac was approved for the prophylaxis of organ rejection in patients receiving allogeneic liver transplants in the United States and later also for use as an immunosuppressant after kidney transplantation.

1.2.2. Physicochemical properties and dosage forms

Tac is a neutral, hydrophobic, macrolide lactone with a hemiketal-masked α, β-diketoamide incorporated in a 23-member ring. Its molecular formula is $C_{44}H_{69}NO_{12}$ with a molecular weight of 804. It appears frequently under its monohydrated configuration. The full chemical name (INN) of Tac is [3S-[3R*[E(1S*,3S*,4S*)],4S*,5R*,8S*,9E, 12R*,14R*,15S*,16R*,18 S*,19S*,26aR*]]-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylethylenyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-8-(2-propenyl)-15,19-epoxy-3H-pyrido[2,1-c][1,4]oxaazacyclotricosine-1,7,20,21(4H,23H)-tetrone,monohydrate. It is soluble in methanol, ethanol, propan-2-ol, acetone, ethyl acetate, acetonitrile, methylene chloride, chloroform, diethyl ether, sparingly soluble in hexane, petroleum ether and insoluble in water (Tanaka *et al.*, 1987).

Tac (Prograf®) is available as 0.5, 1 and 5 mg capsules which is a solid dispersible formulation on hydroxypropyl methylcellulose (a water-soluble polymer). In addition, an

intravenous (IV) solution is available as Prograf® Concentrate for Infusion (5 mg/ml) containing polyoxyethylene hydrogenated castor oil and dehydrated alcohol. It must be diluted in 5 % dextrose or normal saline and administered as a continuous infusion over 24 hours to minimize the nephrotoxicity of the drug. Ointments containing Tac for the topical treatment of skin lesions during autoimmune diseases are under clinical development (Alaiti *et al.*, 1998). Tac must not be used with tubing, syringes or other equipment containing polyvinyl chloride, since it may be adsorbed on the surface of polyvinyl chloride (Taormina *et al.*, 1992), especially at the low dosages used in paediatric patients.

Tac is stable for many months at room temperature as a white crystalline powder, for at least ten days at room temperature and for almost one year at -70ºC when assayed in whole blood (Freeman *et al.*, 1995). It is unstable in alkaline conditions. The UV spectrum is unspecific and presents a maximum absorbance at around 205 nm. The ^{13}C magnetic resonance spectrum (C^2HCl_3) reveals that Tac in solution exists as an equilibrium mixture of 2 isomers (cis and trans form), probably because of a restricted rotation of the amide bond within the macrolide ring.

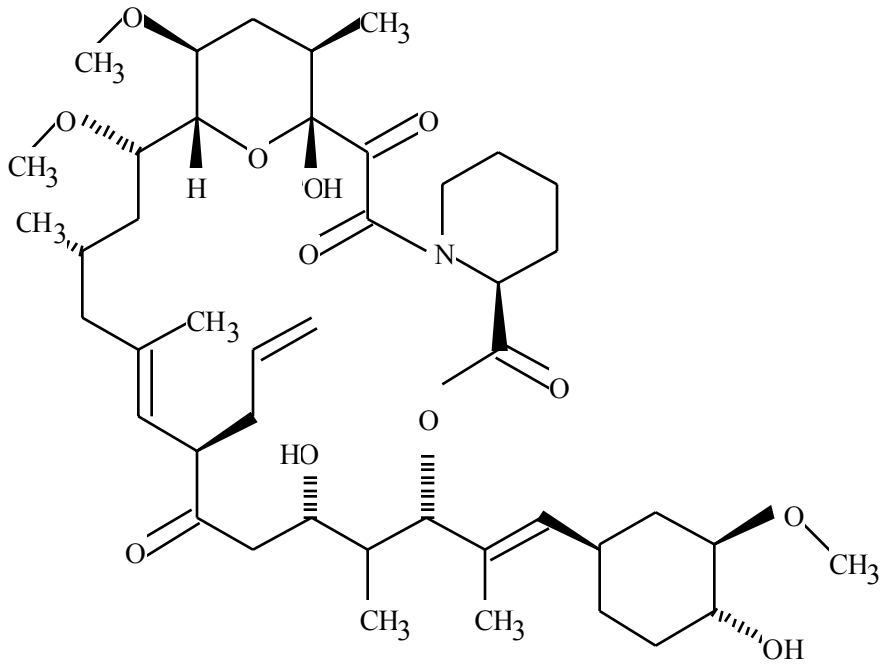


Figure 1. Chemical structure of Tac (FK506).

1.2.3. Mechanism of action and toxicity

Tac prolongs the survival of the host and transplanted graft in animal transplant models of liver, kidney, heart, bone marrow, small bowel and pancreas, lung and trachea, skin, cornea, and limb (Hoffman *et al.*, 1990; Yasunami *et al.*, 1990). In animals, Tac suppresses some humoral immunity and, to a greater extent, cell-mediated reactions such as allograft rejection, delayed type hypersensitivity, collagen-induced arthritis, experimental allergic encephalomyelitis and graft versus host disease.

Tac is a relatively specific inhibitor of lymphocyte proliferation and exerts its immunosuppressive activity mainly through the following mechanisms. Stimulation of

the T cell by an antigen at the T-cell receptor causes phospholipase-mediated production of inositol triphosphate, an increase in cytosolic calcium concentration, formation of an activated calmodulin-calcineurin complex, and activation of a competent transcription factor (nuclear factor of activated T cells [NF-AT]). Tac binds competitively and with high affinity to a 12 k-Da cytosolic receptor (immunophilin) termed as the FK binding protein (FKBP-12) (Siekierka *et al.*, 1989). Studies have shown that FK506 elicits its immunosuppressive activity by inhibiting the cis-trans peptidyl-prolyl isomerase (PPIase) activity of FKBP. The FK506-FKBP complex binds with the catalytic A subunit of calcineurin and in turn inhibits protein phosphatase activity of calcineurin. This prevents dephosphorylation of the cytoplasmic subunit of NF-AT, which otherwise enters the nucleus and activates expression of T cell activation lymphokine genes (Defranco, 1991; Flanagan *et al.*, 1991; Liu *et al.*, 1991; Schreiber and Crabtree, 1992). The net result is the inhibition of T-lymphocyte activation (i.e., immunosuppression).

The use of Tac as an immunosuppressant is mainly limited by its tolerability profile (Winkler and Christians, 1995). Two types of adverse effects must be differentiated: those caused by over immunosuppression and those caused by drug toxicity. Over immunosuppression results in an increased incidence of infectious complications and malignancies, mainly lymphoma, as well as the failure of vaccination. All these are nonspecific effects, and their incidence correlates with immunosuppressive activity and duration rather than with a specific immunosuppressive drug regimen (Plosker and Foster, 2000).

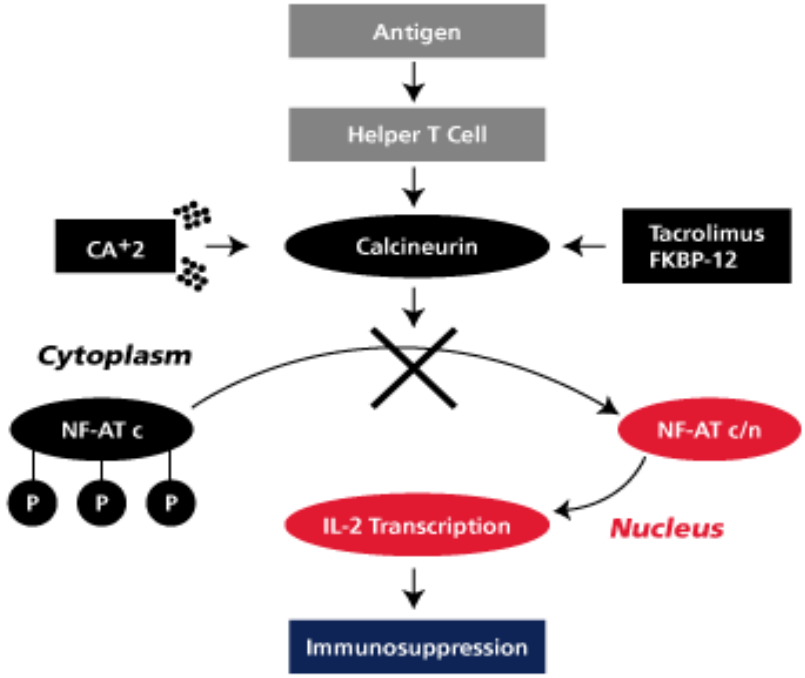


Figure 2. Tac mechanism of action (Fujisawa Healthcare Inc Product Monograph, 2002)

The principal adverse reactions of Tac in major clinical trials are neurotoxicity, diarrhea, hypertension, nausea, and renal dysfunction. These occur with oral and IV administrations of Tac and may respond to a reduction in dosing. The nephrotoxic effect of Tac is not due to a decrease in glomerular filtration rate and renal blood flow but increased renal vascular resistance caused by Tac. The nephrotoxicity may be related to an increased production of thromboxane A_2 production in the renal parenchyma (Yamada *et al.*, 1992).

Diarrhoea was sometimes associated with other gastrointestinal complaints such as nausea and vomiting. Hyperkalemia and hypomagnesemia have occurred in patients

receiving Tac therapy. Tac also has a diabetogenic effect probably due to a change in the islet cells' response to hyperglycemia and a change in peripheral sensitivity of insulin. Some patients may require insulin therapy to overcome the hyperglycemic effect of Tac.

The incidence of major neurological side effect is low (5 %) with Tac and most of them occur during the first month following liver transplant (Eidelman *et al.*, 1991). According to the Pittsburgh study, the patterns and timing of opportunistic infections after surgery are similar under Tac and cyclosporine therapy, occurring early in the post transplant stage (Alessiani *et al.*, 1991).

1.2.4. Analytical methods

Tac concentrations in biological fluids have been measured using a number of methods. The currently available assays can be broadly classified as enzyme immunoassays, chromatographic/mass spectrometric (MS) assays, a radioreceptor assay and bioassay. The analytical methods used for assaying Tac have been reviewed (Alak, 1997). The methods developed for measurement of Tac are summarized in Table 2.

The development of a simple, specific and sensitive assay method for measuring Tac in biological fluids is limited by: the low absorptivity, the low concentration in plasma/blood, and the presence of several other drugs in the blood samples obtained from transplant patients, which potentially interfere with the analysis of Tac.

Table 2. Assays for the quantification of Tac and its metabolites in blood and plasma.

Assay	Matrix	Sensitivity (ng/ml)	CV (%)	Duration of assay (hr)	References
Elisa SPE (Fujisawa)	Plasma	0.1	13-27	36	(Tamura *et al.*, 1987)
Elisa LPE (Fujisawa)	Plasma/ Blood	0.1 / 0.5	10-20	36	(Wallemacq *et al.*, 1993) (Jusko and D'Ambrosio, 1991)
Elisa Pro-Trac (IncStar)	Blood	0.2	5.6-25.5	5	(D'Ambrosio *et al.*, 1994)
Elisa Pro-Trac II (IncStar)	Blood	0.2	6.3-13.1	< 4	(MacFarlane *et al.*, 1996)
MEIA Tac (Abbott)	Blood	5	5.1-7.3	< 0.75	(Grenier *et al.*, 1991)
MEIA Tac II (Abbott)	Blood	1	5-10	< 0.75	(Wallemacq *et al.*, 1997)
HPLC	Blood	3	9.9-11.5	< 5	(Perotti *et al.*, 1994)
HPLC chemiluminescence	Plasma	0.5	8.4	< 5	(Takada *et al.*, 1990)
HPLC fluorescence	Blood	3	10	0.5	(Beysens *et al.*, 1994)
HPLC/ELISA or MEIA	Plasma/ Blood	5	3-16	< 5	(Friob *et al.*, 1991) (Firdaous *et al.*, 1995)
HPLC/MS	Blood	0.2	4.7-15.8	< 3	(Christians *et al.*, 1991)
HPLC/MS/MS	Blood	0.2	< 8	2.5	(Taylor *et al.*, 1996)
T cell inhibition	Plasma	0.1	NA	> 72	(Zeevi *et al.*, 1991)
Radioreceptor assay	Blood	1	8.2-9.2	3-5	(Murthy *et al.*, 1992)
Pentamer formation assay	Blood	2	5.7-13.7	3-5	(Armstrong *et al.*, 1998)
Functional reporter gene assay	Blood	0.5	5.4 -12.7	8	(Taupin *et al.*, 2001)

CV = coefficient of variation; **ELISA** = enzyme-linked immunosorbent assay; **HPLC** = high pressure liquid chromatography; **LPE** = liquid phase extraction; **MEIA** = microparticle enzyme- linked immunoassay; **MS** =mass spectrometry; **NA** = not applicable; **SPE** = solid phase extraction.

1.2.5. Pharmacokinetics

Tac is primarily used in transplant patients who receive an organ that is either involved in the absorption (small bowel) or elimination (liver) of the drug. The physiological status of the organs transplanted is expected to influence the absorption, distribution and metabolism of Tac (Venkataramanan *et al.*, 1995). Time-dependent changes in the absorption, distribution and metabolism of Tac are also anticipated in patients receiving Tac therapy. Tac activity is primarily due to the parent drug (Venkataramanan *et al.*, 1995).

Absorption

Tac is absorbed rapidly in most patients, with peak plasma/blood concentrations being reached in about 0.5 to 1 hour, while in other patients the drug is absorbed slowly over a prolonged period, yielding essentially a flat absorption profile (Gruber *et al.*, 1994). A lag time of 0 to 2 hours has also been reported in some liver transplant recipients (Jusko *et al.*, 1995a). The oral F (bioavailability) of Tac is poor and ranges from 4 to 89 % (mean around 25 %) in kidney and liver transplant recipients and in patients with renal impairment (Gruber *et al.*, 1994).

Because Tac is a substrate of the cytochrome P450 (CYP) 3A4 isoenzyme (Sattler *et al.*, 1992), its poor F is most likely caused by presystemic metabolism in the gut wall and liver. Several studies have shown that Tac is also a substrate of permeability-glycoprotein (P-glycoprotein) efflux pump, the 170-kd product of the human multidrug-resistance 1 (MDR-1) gene, a member of the adenosine triphosphate binding cassette superfamily of active

transporters (Wacher *et al.*, 1995), that is found at the luminal face of enterocytes and that has also been shown to play a role in intestinal absorption mechanisms (Lo and Burckart, 1999). It is, therefore, very likely that the poor and variable F of Tac is at least partly caused by the activity of this efflux pump in the intestine and genetic polymorphism of the P-glycoprotein (Hoffmeyer *et al.*, 2000).

The rate and extent of Tac absorption were greatest under fasted conditions. The presence and composition of food decreased both the rate and extent of Tac absorption (Bekersky *et al.*, 2001). Efforts to increase the oral F of Tac and to reduce its variability include the synthesis of Tac prodrugs (Hiroshi *et al.*, 1999), the development of oral formulations based on liposomes (Lee *et al.*, 1995) and emulsions (Uno *et al.*, 1997).

Distribution and protein binding

Tac is highly lipophilic and undergoes extensive tissue distribution, as evidenced by a large volume of distribution at steady state (V_{SS} ~ 1300 L) estimated from plasma data (Peters *et al.*, 1993). However, V_{SS} estimated from whole blood data was very small (~ 48 L), indicating that there is extensive partitioning into red blood cells. In blood, erythrocytes sequester 75-80 % of the Tac as it has a high affinity for the FK-binding proteins and the abundance of these proteins in erythrocytes and lymphocytes (Nagase *et al.*, 1994). This results in whole blood concentrations of Tac being substantially higher than plasma concentrations. The distribution of Tac between whole blood and plasma depends on several factors, such as hematocrit, temperature at the time of plasma separation, drug concentration, and plasma protein concentration. In plasma, more than 98.8 % of Tac is bound to plasma

proteins, mainly albumin, α_1-acid glycoprotein, lipoproteins and globulins (Nagase *et al.*, 1994). In animal studies, Tac is widely distributed into tissues with the highest accumulation in lung, spleen, kidney, heart, pancreas, brain, muscle and liver (Wijnen *et al.*, 1991).

Metabolism

Tac undergoes extensive hepatic metabolism with < 1 % of unchanged drug being excreted in the bile, urine and faeces after intravenous or oral dosing. Metabolism is mainly by the CYP P450 3A enzyme system (Sattler *et al.*, 1992) and CYP enzymes other than P450 3A have a minor involvement. Tac undergoes O-demethylation, hydroxylation and/or oxidative metabolic reactions. Several metabolites are the product of a two-step reaction: oxidation by CYP enzyme destabilizes the macrolide ring and leads to its rearrangement (Lhoëst *et al.*, 1993). Seven different isomers of 13-O-desmethyl-Tac were detected by using 2-dimensional homo- and heteronuclear magnetic resonance experiments in one study (Schüler *et al.*, 1993).

The role of phase II metabolism in Tac elimination is unclear. It was speculated that early eluting peaks in liquid chromatography after incubation of Tac with rat and human liver slices may represent secondary or conjugated Tac metabolites (Ueda *et al.*, 1996). In liver transplant recipients, the demethylated and didemethylated metabolites were found primarily in the blood and urine, while the hydroxylated metabolites were prominent in the bile (Christians *et al.*, 1991). The Tac metabolites, except for 31-O-desmethyl Tac, which *in vitro* exhibits immunosuppressive activity comparable to that of tacrolimus, have negligible immunosuppressive activity (Tamura *et al.*, 1994). Since 31-O-desmethyl Tac is only a

minor metabolite in blood (Mancinelli *et al.*, 2001), it seems reasonable to assume that the metabolites do not significantly contribute to the overall immunosuppressive activity of Tac (Plosker and Foster, 2000).

Excretion

In healthy subjects, the total body clearance (CL) based on whole blood concentrations was 2.43 L/hr compared with 4.05 L/hr in liver transplant patients and 6.7 L/hr in kidney transplant patients. The elimination half-life ($t_{½}$) based on whole blood concentrations averaged 17.6 hours in healthy volunteers, 11.7 hours in liver transplant patients, and 15.6 hours in kidney transplant patients.

In a mass balance study of IV administered radiolabelled Tac to six healthy volunteers, the mean recovery of radiolabel was 77.8 ± 12.7 %. Faecal elimination accounted for 92.4 ± 1.0 % and the $t_{½}$ based on radioactivity was 48.1 ± 15.9 hours whereas it was 43.5 ± 11.1 hours based on Tac concentrations. The total body CL of radiolabel was 37.2 ± 18 ml/min and that for tacrolimus was 37.5 ± 9.8 ml/min. When administered orally, the mean recovery of the radiolabel was 94.9 ± 30.7 %. Faecal elimination accounted for 92.6 ± 30.7 % and urinary elimination accounted for 2.3 ± 1.1 % (Möller *et al.*, 1999).

1.2.6. Drug interactions

Drug interactions occur when the efficacy or toxicity of a drug is altered by coadministration of another drug. The interaction between Tac and other drugs can be

divided into three categories: (i) physical interactions; (ii) metabolic interactions; and pharmacodynamic interactions.

Physical interactions

Aluminium hydroxide gel appears to physically adsorb Tac *in vitro* (Steeves *et al.*, 1991). This same *in vitro* study also indicated that Tac concentrations are significantly decreased in the presence of magnesium oxide due to a pH-mediated degradation (Steeves *et al.*, 1991). Widely variable trough plasma Tac concentrations were observed in patients taking sodium bicarbonate temporally close to Tac administration. Coadministration of Tac with sodium bicarbonate results in lower blood concentrations of Tac (Venkataraman *et al.*, unpublished observations). Separation of the administration of these 2 agents by at least 2 hours, or the replacement of sodium bicarbonate by sodium citrate and citric acid, results in stable trough plasma Tac concentrations in patients.

Metabolic interactions

Since Tac is metabolized by the CYP450 3A enzyme system, co-administration of drugs which inhibit CYP450 3A will decrease the metabolism of Tac with resultant increases in whole blood or plasma levels (Mignat, 1997). Co-administration of drugs known to induce these enzyme systems may result in an increased metabolism of Tac and decreased whole blood or plasma levels. Monitoring of blood levels and appropriate dosage adjustments are essential when such drugs are used concomitantly. Table 3 shows some agents, which potentially alter the metabolism of Tac.

Pharmacodynamic (PD) interactions

The addition of nephrotoxic drugs such as nonsteroidal anti-inflammatory drugs (Sheiner *et al.*, 1994), aminoglycosides (Paterson and Singh, 1997), or amphotericin B (Paterson and Singh, 1997) to Tac therapy might result in an increased risk of nephrotoxicity in transplant patients and should warrant increased observation of these patients for signs of nephrotoxicity. The combined use of cyclosporine and Tac results in synergistic immunosuppression and increased nephrotoxicity (McCauley *et al.*, 1990).

1.2.7. Tac immunosuppressive therapy optimisation

1.2.7.1 Therapeutic drug monitoring

Therapeutic drug monitoring (TDM) is useful and/or even necessary for dose adjustment to avoid toxicity as a result of too high and ineffectiveness as a result of too low blood concentrations. This is the case when drugs have one or more of the following PK characteristics (Evans *et al.*, 1992):

1) wide inter- and/or intra-individual variations
2) low correlation between dose and blood concentrations
3) difficult to recognize signs of toxicity
4) influence of pathophysiologic factors on PK
5) drug interactions in combinations with narrow therapeutic indices

Drug	Proposed mechanisms	Clinical effect	References
Diltiazem	Inhibition of CYP3A	Increased Tac exposure → toxicity	(Hebert and Lam, 1999)
Mibefradil	Inhibition of CYP3A	Increased Tac exposure → toxicity	(Krahenbuhl *et al.*, 1998)
Fluconazole	Inhibition of CYP3A	Increased Tac exposure → toxicity	(Osowski *et al.*, 1996)
Erythromycin	Inhibition of CYP3A	Increased Tac exposure → toxicity	(Padhi *et al.*, 1997)
Theophylline	Inhibition of CYP3A	Increased Tac exposure → toxicity	(Boubenider *et al.*, 2000)
Danazol	Inhibition of CYP3A	Increased Tac exposure → toxicity	(Shapiro *et al.*, 1993)
Protease inhibitors	Inhibition of CYP3A	Increased Tac exposure → toxicity	(Sheikh *et al.*, 1999)
Carbamazepine	Induction of CYP3A2	Decreased Tac exposure → rejection, no clinical report	(Mignat, 1997)
Rifampicin	Induction of CYP3A4	Decreased Tac exposure → rejection	(Chenhsu *et al.*, 2000)

Table 3. Agents that may alter Tac metabolism.

Tac meets all these requirements indicating that TDM is mandatory. Issues related to TDM of Tac include the appropriateness of whole blood as the matrix in which to measure Tac, the stability of Tac in blood or plasma and the therapeutic range of Tac. A consensus document on therapeutic monitoring of Tac has defined the therapeutic range, matrix, time of sampling, rules for assessing the laboratory performance, and the frequency of TDM (Jusko *et al.*, 1995b). The therapeutic ranges of whole blood Tac levels at various periods post liver transplant, based on this consensus report is shown in Table 4.

Time post-transplant	1 – 4 weeks	1 – 12 months	> 12 months
Tac whole blood trough concentration (ng/mL)	15 – 20	8 – 12	5 - 10

Table 4. Therapeutic ranges of Tac at various periods post liver transplant.

Monitoring of Tac blood concentrations in conjunction with other laboratory and clinical parameters is considered an essential aid to patient management for the evaluation of rejection, toxicity, dose adjustments, and compliance. Factors influencing frequency of monitoring include but are not limited to hepatic or renal dysfunction, the addition or discontinuation of potentially interacting drugs and the post-transplant time (Venkataramanan *et al.*, 1995). For example, monitoring every 1-2 days is required immediately post-transplant due to variable PK and acute infection. For the first 3-6 months, 2-3 times a week until the patient is stable. Beyond 6 months, once every few months, or whenever clinically indicated. In addition, other tests are required to check on the adverse effects of immunosuppressants include creatinine for nephrotoxicity, liver function and

glucose for possible endocrine changes as indicated by either glucose elevation or depression.

Although there is a lack of direct correlation between Tac concentrations and drug efficacy, data from Phase II and III studies of liver transplant patients have shown an increasing incidence of adverse events with increasing trough blood concentrations (Schwartz *et al.*, 1995). In a retrospective analysis of 13,000 blood samples obtained from 248 liver and kidney transplant patients receiving Tac immunosuppressive therapy, Winkler *et al.* reported that whole blood Tac concentrations correlated with clinical events related to organ rejection and toxicity (Winkler *et al.*, 1994). Most patients are stable when trough whole blood concentrations are maintained between 5 and 20 ng/mL (Venkataramanan *et al.*, 1995). Long-term post-transplant patients are often maintained at the low end of this target range. In a retrospective analysis of trough whole blood concentrations within a 7-day window before the onset of rejection, blood concentrations were well correlated with the onset of rejection (Kershner and Fitzsimmons, 1996).

In an investigation to determine the clinical utility of monitoring Tac blood concentrations in liver transplant patients involving 111 subjects from 6 transplant centres evaluated over 12 weeks post-transplantation, Tac blood concentrations were shown to provide information of predictive value for managing the risk of nephrotoxicity, other toxicity, and rejection in liver transplant patients (Venkataramanan *et al.*, 2001). In paediatric liver transplant patients, rejection was shown to be most frequent at blood concentrations less than 10 ng/mL (Yasuhara *et al.*, 1995). In a retrospective PD investigation on 35 paediatric

liver recipients who received oral Tac as the primary immunosuppressant, a statistically significant relationship exists between some Tac toxicities (nephrotoxicity, neurotoxicity and diarrhoea) and tacrolimus trough concentrations (Staatz *et al.*, 2004). To minimize toxicity in the later post-transplant period, it was proposed a target trough Tac concentration of 6 ng/mL be reached by the authors.

A new strategy for drug optimisation in transplant recipients is represented by dose adjustment on the basis of drug target activity. Recent studies have evaluated the effects of Tac on calcineurin phosphatase activity in blood (Koefoed-Nielsen *et al.*, 2002). Plasma or blood concentrations of Tac did not correlate with degree of calcineurin inhibition, while most studies show a wide interpatient variability of drug effects. Although this strategy for drug optimisation needs to be further investigated, it may prove effective to improve dose optimisation of Tac in individual transplant recipients.

In conclusion, it can be seen that monitoring Tac blood concentrations are useful for minimizing the risks of both rejection and toxicity in liver transplant patients. Routine monitoring of Tac blood concentrations must be used in conjunction with appropriate clinical evaluation of the patient to optimise immunosuppressive therapy.

1.2.7.2 Pharmacogenomics and pharmacogenetics

The advent of the genomic era has brought several new fields of study, including pharmacogenomics, which seek to link drug treatment with the individual's genetic makeup.

Thus it represents a new approach to tailor immunosuppressive therapy during organ transplantation (Danesi *et al.*, 2000). Pharmacogenomics and the much older science of pharmacogenetics examine the genetic factors that contribute to variability in drug response in individual patients. Specifically, pharmacogenetics refers to the field science that focuses on how single genes modulate the effect of a drug while pharmacogenomics refers to the science that focuses on how the genome as a whole affects the action of a drug, referring to the contribution of individual genes, as well as to gene-to-gene interactions (Yagil and Yagil, 2002).

In recent years, much research has focused on the possible causes of the interindividual and intraindividual differences in the PK of Tac. It has become clear that the biologic activities of the permeability-glycoprotein (P-glycoprotein) and the CYP450 enzyme system, especially CYP3A4 and CYP3A5, play an important role in this respect (Shimada *et al.*, 1994). Part of the interindividual differences in the PK of Tac have been attributed to interindividual heterogeneity in enzymatic activity of P-glycoprotein and CYP3A. However, the cause of this heterogeneity in enzymatic activity remains to be elucidated.

Recently, a number of single nucleotide polymorphisms (SNPs), which are common variations in the structure of a gene and consist of one-nucleotide alteration in the sequence of a gene (McCarthy and Hilfiker, 2000), were described for the MDR-1 (Hoffmeyer *et al.*, 2000; Kim *et al.*, 2001; Tanabe *et al.*, 2001), CYP3A4 (Rebbeck *et al.*, 1998; Sata *et al.*, 2000), and CYP3A5 (Jounaidi *et al.*, 1996; Hustert *et al.*, 2001; Kuehl *et al.*, 2001) genes. The C3435T mutation in the MDR-1 gene has been associated with decreased protein

expression, whereas the CYP3A5*3 and CYP3A5*6 alleles were found to cause alternative splicing and protein truncation, resulting in the absence of functional CYP3A5 from liver tissue (Hustert *et al.*, 2001; Kuehl *et al.*, 2001). For the CYP3A4*1B allele, an increased transcription was demonstrated *in vitro*, which would theoretically result in higher enzymatic activity *in vivo* (Amirimani *et al.*, 1999). Therefore these genetic polymorphisms may provide an explanation for the observed variability in Tac PK.

The polymorphisms associated with the expression of CYP3A5 seem to have a greater importance than those of CYP3A4. A study of paediatric heart transplant patients (Zheng *et al.*, 2003) demonstrated that the dose requirements for Tac were higher in expressors (genotype, CYP3A5 1*/3*) compared with non-expressors (genotype, CYP3A5 3*/3*) at 3, 6 and 12 months post-transplantation. Macphee *et al.* (Macphee *et al.*, 2002) described similar Tac dose requirement patterns for adult renal transplant patients. The authors suggested that the P4503AP1*1 genotype was a major influence in determining Tac dose requirements. This polymorphism, at position 44 of the P4503AP1 pseudogene (P4503AP1*1 or G-allele) is in linkage disequilibrium with CYP3A5*1. It results in either the presence or absence of P4503A5 enzyme (Kuehl *et al.*, 2001; Lin *et al.*, 2002). Individuals with a G-allele (P4503AP1*1) express hepatic CYP3A5 enzyme (Kuehl *et al.*, 2001; Lin *et al.*, 2002), whereas AA homozygotes (P4503AP1*3/*3) do not. Thervet *et al.* reported the significant effect of CYP3A5 genetic polymorphism on Tac dosing requirements in renal transplant recipients (Thervet *et al.*, 2003). Their results in a cohort of 80 renal transplant recipients show that the *1/*1 variant (5% of the population) required a lower mean dose of Tac to achieve trough concentrations of 10 to 15 ng/mL compared with the

*3/*3 homozygotes (84% of the population) (0.16 mg/kg/day vs. 0.25 mg/kg/day, P=0.05). The *1/*1 genotype was found mostly in black patients, which corresponds with previously identified ethnic differences, with African Americans known to require higher doses (Andrews *et al.*, 1996).

Several polymorphisms have been found for the MDR-1 gene (Hoffmeyer *et al.*, 2000; Kim *et al.*, 2001; Tanabe *et al.*, 2001). Of these, three mutant alleles on exons 12, 21 and 26 correlate with expression of the MDR-1 gene and function of P-glycoprotein. The G2677[A/T] SNP was shown to influence blood concentrations of Tac in paediatric heart transplant patients (Zheng *et al.*, 2003), but not in liver transplant recipients (Goto *et al.*, 2002). The C3435T SNP was shown to affect Tac dose requirements in renal transplant patients (Macphee *et al.*, 2002).

Thus it can be seen that there is scope for genetic analysis to be used in the clinical field at present, with pretransplant testing of genes allowing a more scientific dosing in the first few days posttransplant. The initial aim of this should be to identify those patients with increased drug requirements to avoid underimmunosuppression. It may also be possible to identify groups of patients who are more likely to develop drug-related side effects at therapeutic doses. With clearer understanding of the interactions involved, it may be possible to predict individual dose requirements and responses.

1.3 Literature review

A literature review of the PK studies of Tac in adult and paediatric transplant patients was conducted and the results summarized in Tables 5 and 6, respectively. From the results, it can be seen that there are more PK studies of Tac carried out in adult transplant patients than in paediatric patients. Also, most of the studies were conducted in Caucasian patients. Thus, there is a need for PK studies to be conducted in the asian paediatric transplant patients to provide population mean parameters required for dosage adjustments of Tac immunosuppressive therapy in this group of patients.

Table 5. PK parameter values of Tac in adult transplant recipients. Values were reported from whole blood samples.

Trans-plant type	n	Age (year)	Race	Route	Dose (mg/kg/day)	F (%)	V_{SS} (L/kg)	CL (L/h/kg)	C_{max} (ng/mL)	C_{min} (ng/mL)	AUC_{12} (ng• h/mL)	Reference
Liver	16	33-35	Caucasian	IV	0.014 – 0.11		0.906±0.29	0.0541±				(Jusko *et al.*,
				Oral	0.1 – 0.3	25±10		0.0173				1995a)
Liver	35	15-61	Asian	IV	0.013±0.007		1.52	0.021[a]				(Fukatsu *et al.*,
				Oral	0.056±0.086	6.8						2001)
Liver	9	24-64	Caucasian	IV	0.15		17.6±9.4	1.49±1.48				(Jain *et al.*,
												1993)
Liver	7	20-61	Caucasian	Oral	0.08±0.04				17.4±6.4		328±280	(Gonschior *et*
												al., 1994)
Renal	18	43±14	Caucasian	Oral	0.08±0.07						116±50	(Hardinger *et al.*,
												2004)
Renal	15	21-56	Caucasian	Oral	0.3				43.7(126%)	13.6(69%)	254 (75%)	(Higgins *et al.*,
												2000)
Renal	19	43.6±10.4	Caucasian	IV	0.037±0.01		1.42± 0.57	0.0060±			408±157	(Tuteja *et al.*,
				Oral	0.13±0.07	19.1±10		0.002			203±66	2001)
Renal	22	35.7±10.1	Caucasian and non-caucasian	Oral	0.13±0.04				28.4±9.4	11.6±4.9	198.2±68	(Felipe *et al.*, 2002)
Renal	17[b]	38.2±13.7	Asian	Oral	0.19±0.07				63.2±10.9	17.7±6.4	304.4±54.8	(Kimikawa *et*
	16[c]	34.5±10.6			0.15±0.06				34.7±12.8	19.5±6.8	262.1±97.1	*al.*, 2001)

Trans-plant type	n	Age (year)	Race	Route	Dose (mg/kg/day)	F (%)	V_{SS} (L/kg)	CL (L/h/kg)	C_{max} (ng/mL)	C_{min} (ng/mL)	AUC_{12} (ng•h/mL)	Reference
Renal	100	51.4±13.8	Caucasian	Oral	0.14±0.0.06				22.7±8.4	9.85±3.1	160.5±47.9	(Kuypers *et al.*, 2004)
Bone marrow	122	13-60	Caucasian	IV Oral	0.03 0.12	 28±16		0.075± 0.0002[d]				(Jacobson *et al.*, 2001)
Bone marrow	31	32-50	Caucasian	IV Oral	0.026-0.03 0.052-0.063	 31-49	1.67±1.02	0.071±0.034	 44.6±34.4		 406±395	(Boswell *et al.*, 1998)
Heart	11	>18	Caucasian	Oral	0.053±0.031				23.6±22.4			(Undre *et al.*, 1998)
Heart	22	36-64	Caucasian	Oral	0.3				30.5±13.8		236.3±88.6	(M D Aumente Rubio *et al.*, 2003)
Heart	14	23-61	Caucasian	Oral	0.06-0.8				10.7-84.4	<5-54.1	103.5-728.7	(Regazzi *et al.*, 1999)

[a] Based on an adult recipient with living-donor liver transplantation, with grafted hepatic weight of 600 g and normal hepatic and renal function on post-operative day = 30.
[b] Preprandial group
[c] Postprandial group
[d] Based on an adult recipient with 70 kg body weight, total bilirubin < 2 mg/dL, serum creatinine < 2 mg/dL, graft-versus-host disease grade I or II and absence of veno-occlusive disease.
AUC_{12} = area under the concentration-time curve from 0 to 12 hr; **C_{max}** = maximum drug concentration; **C_{min}** = minimum drug concentration.

Table 6. PK parameter values of Tac in paediatric transplant recipients. Values were reported from whole blood samples.

Trans-plant type	n	Age (year)	Race	Route	Dose (mg/kg/day)	F (%)	V_{SS} (L/kg)	CL (L/h/kg)	C_{max} (ng/mL)	C_{min} (ng/mL)	AUC_{12} (ng• h/mL)	Reference
Liver	12	0.7-13	Caucasian	IV	0.037±0.013	25±	2.6±2.0	0.138±0.07			1278.4±193.1	(Wallemacq *et al.*, 1998)
	16			Oral	0.152±0.015	20	9.0±11.1		37±26.5		252.4±167.4	
Liver	33	0.25-15	Asian	IV/oral	0.06/0.30	19± 4	2.76[a]	0.075[b]				(Yasuhara *et al.*, 1995)
Liver	18	0.25-16	Caucasian	Oral	0.21 (0.06-0.44)			0.21[c]				(Sanchez *et al.*, 2001)
Renal	14	5-23	Caucasian	Oral	0.16 (0.04-0.30)				15.2±6.7	7.1±2.6	104±33	(Filler *et al.*, 1997)
Renal	32	3-15	Asian	IV	0.078±0.018	10.0±		0.126±0.036			335±79	(Shishido *et al.*, 2001)
				Oral	0.60±0.16	5.2			26.2±8.3		212±50	
Bone marrow	7	8-17	Caucasian	IV	0.03-0.04			0.09(0.03-0.15)[d]				(Mehta *et al.*, 1999)
				Oral	0.12-0.16			0.108(0.08-0.14)				

a V estimated for 15 kg according to: V (L) = 2.76•(TBW/15)$^{0.29}$ and TBW is total bodyweight.
b CL estimated for 15 kg and days after operation (POD) = 0 according to CL (L/h) = (0.0749 + 0.000457•POD)•size when size = 2.76•(TBW/15)$^{0.29}$.
c CL estimated for 15 kg according to CL (L/h) = 10.4•(TBW/70)$^{0.75}$ •$e^{-0.00032\,t}$ • $e^{-0.057\,BIL}$ (1-0.079ALT) when time after treatment initiation (t) = 0, bilirubin (BIL) = 0.6 mg/dL and normal alanine aminotransferase (ALT = 0).
d CL decreases with time.
AUC_{12} = area under the concentration-time curve from 0 to 12 hr; **C_{max}** = maximum drug concentration; **C_{min}** = minimum drug concentration.

RESEARCH GOAL AND OBJECTIVES

2. RESEARCH GOAL AND OBJECTIVES

The overall goal of our research is to optimise the use and patient outcomes of Tac immunosuppressive therapy in liver transplantation.

The specific objectives are:

(1) To retrospectively determine the population PK of oral and IV Tac in local Asian paediatric liver transplant recipients, which represents a homogeneous subpopulation with special PK characteristics.

(2) To prospectively determine the population PK of oral Tac in the local Asian liver transplant recipients, comprising of a more heterogeneous group of paediatric and adult patients using a bioanalytical assay to measure blood concentrations of Tac.

For the determination of the population PK of Tac, the data were analyzed with a systematic approach to screen covariates for inclusion into the population model and the final population models were evaluated.

POPULATION PHARMACOKINETICS OF TACROLIMUS IN ASIAN PAEDIATRIC LIVER TRANSPLANT PATIENTS

3.1. Study Aims

The PK of Tac have been investigated extensively in adult liver transplant patients (Jain *et al.*, 1993; Lee *et al.*, 1993; Mekki and Lee, 1994; Undre and Möller, 1994; Jusko *et al.*, 1995a). However, there is limited data on the PK of Tac in paediatric liver transplant patients (Yasuhara *et al.*, 1995; Wallemacq *et al.*, 1998). This hampers the development of optimal treatment protocols, individualisation of doses and successful utilisation of therapeutic drug monitoring in paediatric transplant patients. These could affect the clinical outcomes especially since Tac has a narrow therapeutic index, a large variation in PK within and between individuals; is subject to a range of metabolic drug interactions involving cytochrome P450; and produces adverse effects such as nephrotoxicity and neurotoxicity (Venkataramanan *et al.*, 1995). Thus the paucity of age-specific PK data has caused difficulty in defining the optimum dosage regimen and has highlighted the usefulness of TDM.

The technique of population PK was developed for the interpretation of the limited data available from a wide range of patients, rather than the extensive data from few subjects generated in typical PK studies (Whiting *et al.*, 1986). By definition, population PK is the study of the PK similarities and differences between individuals from measurements of drug levels in biological fluids of subjects or patients arising from some population of interest (Aarons, 1991).

The nonlinear mixed-effects model (NONMEM) population PK program (Boeckmann *et al.*, 1992) was used to assess information regarding the PK profile of Tac in this fragile population. It allows for the development of a complete population PK model, including average PK parameters, covariates and the intra- and inter-individual variabilities. Such modeling is especially useful when there are only a few PK measurements from each individual sampled in the population, or when the data collection design varies considerably between these individuals. Results from a population analysis will provide specific subpopulation parameters to be used in predictions. This is in contrast to using general population parameters, which may be inappropriate to achieve the target whole blood Tac concentration range of 10-20 ng/mL, a concentration range that has been found to be therapeutic in paediatric liver transplant inpatients after living-related liver transplant (Yasuhara *et al.*, 1995).

The purpose of this study was to investigate the disposition of Tac in a group of sixteen Asian paediatric liver transplant patients using a population PK analysis, and to elucidate any significant clinical or demographic effects that might necessitate dosage adjustments. To verify the predictive performance of the population PK model, subsequent Tac blood concentrations were predicted for an independent group of four patients and then compared with the measured concentrations.

3.2. Methods

Tac assay

Whole blood concentrations of Tac were measured by a competitive binding microparticle enzyme immunoassay (MEIA) with a procedure using an Abbott IMx analyser (Abbott®, Chicago, IL, U.S.A.) (Grenier *et al.*, 1991) by the hospital's clinical laboratory. The lower limit of quantification of the assay was 5 ng/mL and it was linear between 4.5 ng/mL to 30.0 ng/mL. The between-day coefficient of variation (CV) of the assay was 16.0 % at a concentration of 5 ng/mL, 9.0 % at a concentration of 10 ng/mL, and 10.0 % at a concentration of 20 ng/mL. The within-day CV of the assay was 8.7 % at a concentration of 5 ng/mL, and 5.0 % at the concentrations of 10 ng/mL and 20 ng/mL.

Population PK Modeling

The PK of Tac was determined using a population approach in which concentrations from all patients were analysed simultaneously to produce estimates of the PK parameters. In general terms, a population model consists of three sub-models. The structural model relates the independent variable, for example time, to the observations (e.g. drug concentrations in the blood). The covariate model relates the available demographic information such as age, gender and clinical laboratory measurements to the parameters of the structural model. The statistical model describes the differences between (1) the parameter values of the typical individual in the population under study

to the individual values of the parameters and (2) the observations and the model predictions, which are the residuals (Boeckmann *et al.*, 1992).

Blood concentration-time profiles in the database were used for non-linear mixed effect modeling by extended least squares regression using the NONMEM computer program (Version IV, level 2.0), with double precision (Boeckmann *et al.*, 1992), installed on a Pentium II 300 MHz personal computer (Aris, Exprez-series). Fortran subroutines were complied under Microsoft FORTRAN Powerstation (Version 4.00).

The first-order estimation method was used to estimate population mean parameters, intersubject variability in these parameters and residual intrasubject variability between observed and predicted Tac concentrations. The concentration-time course of Tac was described by using a one-compartment model with first-order absorption and elimination. The model was parameterized in terms of CL, V and F and implemented using the data preprocessor NMTRAN and PREDPP. The absorption rate constant, k_a of the model was fixed at 4.5 hr^{-1}, as a result of a sensitivity analysis carried out to determine the value of k_a to be used in the NONMEM analysis.

Pharmacostatistic modeling

A constant coefficient of variation model was used to describe the deviations of Tac CL (CL_j), V (V_j) and F (F_j) of the jth individual from the true (but unknown) population mean values.

$$CL_j = TVCL \bullet (1 + \eta_{j,CL})$$

$$V_j = TVV \bullet (1 + \eta_{j,V})$$

$$F_j = TVF \bullet (1 + \eta_{j,F})$$

TVCL, TVV and TVF are the population mean values for CL, V and F, respectively. $\eta_{j,CL}$, $\eta_{j,V}$ and $\eta_{j,F}$ are random variables that distinguish the jth individual's parameter from the population mean as predicted by the regression model and are assumed to be independent and normally distributed with mean zero and variance ω^2. The magnitude of interpatient variability in the structural parameters was expressed as a CV.

An additive model was used to describe the residual error ($\in$) between the observed (C) and the predicted (C_{pred}) concentrations for the jth individual. These differences ($\in_{ij}$) are attributable to intrapatient variability in PK parameters over time, assay error, sampling and dosing time errors, interoccasional variability, misspecification of the PK model and the influences of covariates which are either not included, or unknown.

$$C_{ij} = C_{pred,ij} + \in_{ij}$$

C_{ij} is the ith observed concentration for the jth individual. $C_{pred,ij}$ is the blood Tac concentration predicted by the PK model. $\in_{ij}$ is a randomly distributed variable with zero mean and variance of σ^2. The magnitude of residual variability was expressed as a standard deviation (s.d.).

Regression model

The initial analysis for the population PK of Tac was conducted without including any patient covariates in the model (BASE 1). The value of the objective function (OBJF) from this model was then used as the reference in the subsequent univariate analyses whereby the influence of the patients' covariates on CL, V and F was individually assessed. The regression relationship for CL was modeled as follows for continuous covariates:

$$TVCL = \Theta_{CL} \cdot (\text{covariate/median})$$

$$TVCL = \Theta_{CL} \cdot (\text{covariate/median})^{\Theta_{CL}^{COV1}}$$

$$TVCL = \Theta_{CL} + \Theta_{CL}^{COV1} \cdot (\text{covariate} - \text{median})$$

and as follows for dichotomous covariates (assigned a value of 0 or 1):

$$TVCL = \Theta_{CL} \cdot (1 - \Theta_{CL}^{COV1} \cdot \text{covariate})$$

with Θ_{CL}^{COV1} representing the regression coefficients to be estimated by the NONMEM analysis.

The following potential covariates were screened: patient age (AGE); weight (WT); height (HT); body surface area (BSA); gender (GEN); number of post-operative days (POD); alkaline phosphatase (APH); alanine amino transferase (ALT); aspartate amino transferase (AST); gamma glutamyl transferase (GGT); total bilirubin (TOTBIL);

albumin (ALB); total protein (PROT); lactate dehydrogenase (LDH); serum creatinine (CREA); serum urea (UREA) and haematocrit (HCT). These covariates were chosen as they represent clinical indicators of patient demographics, liver function and renal function. The models for the effect of the covariates on V and F were analogous to those for CL.

Statistical analysis

For each model, the improvement in the fit obtained on addition of a fixed effect variable into the overall model was assessed by several means. Hierarchic models were compared statistically using a likelihood ratio test. The change in the objective function value (ΔOBJF) produced by the inclusion of a covariate represents a statistic which is proportional to minus twice the log-likelihood of the data and approximates a χ^2 distribution with degrees of freedom equal to the difference in the number of structural parameters (i.e. θs) between two models (Neyman and Pearson, 1933). For non-hierarchical models, the comparison was based on direct comparison of OBJF values and on visual inspection of the residual plots. A change in OBJF of $\geq$ 7.88 is required to reach statistical significance ($p = 0.005$) for the addition of one fixed effect (Neyman and Pearson, 1933).

The goodness-of-fit of each model was also assessed by the examination of the scatterplot of weighted residuals vs predicted Tac concentrations, the precision of the

parameter estimate (i.e. % standard errors of the mean and 95 % confidence interval) and by the magnitude of the interpatient and residual variability.

As a result of the univariate analysis, each model with significant effect (i.e. which satisfied all the above-mentioned criteria) was ranked according to its ΔOBJF compared with BASE 1. The model with the largest ΔOBJF was designated as BASE 2 and multiple regression analysis with forward selection was performed where covariates were incorporated into BASE 2 one by one, starting with the second largest ΔOBJF and continuing along the rank order established in the univariate analysis. Each covariate that caused a ΔOBJF of ≥ 7.88 was kept in the model. The model, BASE 3, containing all significant covariates was then subjected to stepwise, backwards elimination where each coefficient for a covariate was set to zero, in turn; the final model contained only those covariates whose omission caused an increase in OBJF of ≥ 7.88.

Model evaluation

To verify the predictive value of the population model, the measured Tac concentrations in the validation group (n = 4) were compared with the corresponding predicted values by the population model using posthoc Bayesian forecasting. This was achieved by fixing the structural and variance model parameters to the values estimated in the final population model.

The predictive performance of the model was assessed in terms of bias (mean prediction error, ME) and precision (root mean square prediction error, RMSE) and the associated 95 % confidence intervals(Sheiner and Beal, 1981). Weighted residuals were plotted against the predicted concentrations to visually assess the deviations among pairs of model-predicted and observed blood Tac concentrations in the validation group.

3.3. Results

Study population and data collection

Data were obtained retrospectively from medical records and routine Tac monitoring of paediatric patients who underwent orthotopic liver transplantation between Jun 1996 and Aug 1999 at the National University Hospital, Singapore. Information extracted from the medical records included date, time, postoperative day, dose of Tac, Tac blood concentration, age, height, current body weight, gender, liver function indices, renal function indices and haematocrit. The information was checked as thoroughly as possible for accuracy. To ensure the accuracy of the data, dosing information and laboratory results as recorded on the patient's medical records were confirmed by referring to the original medicine administration record and clinical laboratory results, respectively.

The total number of patients included in this analysis was based on the availability of patients who underwent orthotopic liver transplantation between Jun 1996 and Aug

1999 at the National University Hospital, Singapore. Patients were assigned randomly to either an index group (n = 16) for development of the PK model(s), or to the validation group (n = 4) for the purpose of assessing the predictive performance of the derived model(s) with characteristics as shown in Table 7. The choice for the number of patients to be included in the validation group is based on the accepted practice of including half to one-third the number of patients in the index group in population modeling.

Tac administration

In four patients, oral Tac was given preoperatively at a dose of 0.3 mg/kg/day for one day. Postoperative immunosuppressive therapy consisted of a combination of steroids and Tac. In ten patients, Tac was administered IV intraoperatively at a dose of 0.05 mg/kg, which was followed postoperatively by a 24 hr continuous IV infusion at an initial dose of 0.05 mg/kg/day for 3-7 days. In ten patients, Tac was commenced enterally via a nasogastric tube starting on the second postoperative day. When oral intake was started, it was given at a dose of 0.2 mg/kg/day in two divided doses. Dosing adjustments were made based on patient responses, adverse effects and the trough drug concentrations.

Patients	Index group	Validation group
Number (M/F)	16 (8/8)	4 (2/2)
Age (yr)	3.7 (1.1 – 13.9)	2.0 (1.2 – 4.0)
Height (cm)	84.3 (68 – 116)	80.5 (73 – 98)
Weight (kg)	12.0 (6.9 – 20.5)	10.3 (8.0 – 14.6)
Race		
Chinese	10	4
Malay	4	
Indian	2	
Tac		
Blood concentration (ng/mL)	12.7 (5.0 – 49.8)	12.7 (5.0 – 30.0)
Number of samples	771	86
Median samples per patient	44 (19-80)	20 (6-40)

Table 7. Characteristics of patients included in the study. Results are presented as the number, or mean (range).

Pharmacostatistic modeling

In the initial model, the data were better described with a one-compartment model with first-order absorption and elimination (subroutines ADVAN 2 TRANS 2) than onewithout considering first-order absorption (subroutines ADVAN 1 TRANS 2), as noted by a greater reduction of the OBJF of 27.0 ($p < 0.001$) and an improvement in the precision in the estimation ω^2_F (coefficient of variation decreases from 422.8 % to 48.3 %).

The one-compartment with first-order absorption and elimination model after oral drug administration is described by the following formula:

$$C_p = \frac{F \times D \times k_a}{V \times (k_a - k_e)} (e^{-k_e t} - e^{-k_a t}) + C_0 \times e^{-k_e t}$$

where k_a is the absorption rate constant, k_e the elimination rate constant, D the dosage given to the patient, t the time, C_0 the initial concentration, and C_p the predicted concentration. A diagrammatic representation of this PK model is shown in Figure 3.

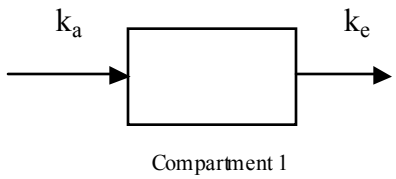


Figure 3. A schematic representation of the one-compartment with first-order absorption and elimination PK model.

As most of the kinetic data were collected in the postabsorption phase, k_a could not be reliably estimated; for this reason, its value was empirically estimated and then fixed throughout the analysis. A sensitivity analysis showed that the lowest estimate errors for the parameters were obtained by fixing k_a to a value of 4.5 hr^{-1} after testing in the basic model with k_a values increasing in interval of 1 from 0.5 to 7.5 hr^{-1} (Table 8).

For the residual variability, the OBJF with use of an additive, slope/intercept and power error models was 1016 lower than that with use of proportional and exponential error models. However, the standard errors of parameter estimates were higher for the slope/intercept and power models (Table 9). Thus an additive error model for residual variability was used in the basic PK model (BASE 1) to be used for further analysis.

Regression models

Results of the univariate analyses showing covariates with significant effects on CL, V and F of Tac in paediatric patients are presented in Table 10. The regression relationship for TOTBIL on F was estimated initially using models for a continuous covariate, the best of which satisfied four out of the five criteria for model discrimination. Thus a categorical "cutoff" model was used, which caused the model to satisfy all the criteria used in model discrimination. Hence, this relationship was used to model the effect of TOTBIL on F. The covariate model causing the largest reduction in OBJF (i.e. linear model of weight on F) was declared as BASE 2, which acted as a reference model for the subsequent analysis of building a more complex model.

k_a (hr^{-1})	Min. obj. fn.	Std. error of parameter estimation (CV%)						
		θ_{CL}	θ_V	θ_F	ω^2_{CL}	ω^2_V	ω^2_F	σ^2
0.5	-6939.836	24.3	58.7	27.7	44.0	91.0	49.4	18.4
1.5	-6962.150	23.4	49.4	26.3	44.2	67.1	48.7	18.9
2.5	-6966.575	23.2	48.1	26.2	44.3	65.1	48.4	19.0
3.5	-6967.900	23.2	47.7	26.0	44.1	64.8	48.2	19.0
4.5	-6968.367	23.2	47.4	25.9	44.1	64.8	48.2	19.1
5.5	-6968.551	23.2	47.4	26.0	44.1	65.0	48.4	19.1
6.5	-6968.632	23.2	47.7	25.9	44.3	64.8	48.1	19.1
7.5	-6968.672	23.2	47.7	26.0	44.3	65.1	48.3	19.1

Table 8. Sensitivity analysis of k_a values used in ADVAN 2 TRANS 2 subroutine.

Table 9. Comparison of interpatient and intrapatient random effects models.

Model			Std. error of parameter estimation (CV%)								
Interpatient	**Intrapatient**	**Min. obj. fn.**	θ_{CL}	θ_V	θ_F	θ_6	ω^2_{CL}	ω^2_V	ω^2_F	σ_1^2	σ_1^2
Additive[a]	Additive[c]	-6982.284	20.3	39.9	24.4		44.5	57.7	51.2	19.0	
Additive	Proportional[d]	-5952.650	20.1	23.0	25.0		38.8	71.5	562.3	28.7	
Additive	Slope/intercept[e]	-6982.284	20.3	39.9	24.4		44.6	57.5	51.0	2.3×10^7	19.0
Additive	Exponential[f]	-5952.650	20.1	23.0	25.0		38.8	71.5	562.3	28.7	
Additive	Power[g]	-6982.284	19.9	39.9	24.3	2.96×10^8	44.5	58.3	50.7	102.7	
Proportional[b]	Additive	-6968.367	23.2	47.4	25.9		44.1	64.8	48.2	19.1	
Proportional	Proportional	-5952.571	20.2	21.9	29.0		22.8	38.8	1.47×10^8	26.0	
Proportional	Slope/intercept	-6968.367	23.2	47.7	26.0		44.1	64.8	48.0	4.7×10^5	19.1
Proportional	Exponential	-5952.571	20.2	21.9	29.0		22.8	38.8	1.47×10^8	26.0	
Proportional	Power	-6968.367	23.2	47.7	25.9	6.76×10^4	44.1	64.8	47.9	19.1	

[a] $P_j = P_{TV} + \eta_j$; [b] $P_j = P_{TV} \times (1 + \eta_j)$; [c] $C_{ij} = C_{pred,\,ij} + \varepsilon_{ij}$; [d] $C_{ij} = C_{pred,\,ij} \times (1+ \varepsilon_{ij})$; [e] $C_{ij} = C_{pred,\,ij} \times (1+ \varepsilon_{1,\,ij}) + \varepsilon_{2,\,ij}$; [f] $C_{ij} = C_{pred,\,ij} \times EXP(\varepsilon_{ij})$; [g] $C_{ij} = C_{pred,\,ij} + C_{pred,\,ij}^{\theta 6}.\varepsilon_{ij}$

Table 10. Summary of univariate analysis showing covariate models with significant effects on CL, V or F of Tac.

Effect on	Model	ΔOBJF [a]	Parameter estimate	95% CI [b] lower	upper	p value
CL [d]	$\Theta_{CL} + \Theta_{CL}^{AGE1} * (AGE-2.25)$ (1 DF)	-19.842	$\Theta_{CL} = 1.48$	0.93	2.03	< 0.001
			$\Theta_{CL}^{AGE1} = 0.294$	0.05	0.54	
V [e]	$\Theta_V + \Theta_V^{WT1} * (WT-11.4)$ (1 DF)	-14.68	$\Theta_V = 45.4$	26.6	64.2	< 0.001
			$\Theta_V^{WT1} = 6.48$	3.79	9.17	
	$\Theta_V + \Theta_V^{BSA1} * (BSA-0.49)$ (1 DF)	-16.83	$\Theta_V = 45.4$	26.9	63.9	< 0.001
			$\Theta_V^{BSA1} = 220$	115.3	324.7	
	$\Theta_V * (HCT/31.9)^{\Theta_V^{HCT1}}$ (1 DF)	-14.421	$\Theta_V = 46.7$	6.13	87.3	< 0.001
			$\Theta_V^{HCT1} = -0.688$	-1.13	-0.25	
F [f]	$\Theta_F * (AGE/2.25)^{\Theta_F^{AGE1}}$ (1 DF)	-16.271	$\Theta_F = 0.178$	0.11	0.25	< 0.001
			$\Theta_F^{AGE1} = 0.484$	0.20	0.76	
	$\Theta_F + \Theta_F^{WT1} * (WT-11.4)$ (1 DF)	-36.574	$\Theta_F = 0.185$	0.13	0.24	< 0.001
			$\Theta_F^{WT1} = 0.0204$	0.01	0.03	
	$\Theta_F + \Theta_F^{HT1} * (HT-82)$ (1 DF)	-23.518	$\Theta_F = 0.187$	0.12	0.26	< 0.001
			$\Theta_F^{HT1} = 0.00609$	0.001	0.011	
	Θ_F IF TOTBIL ≥ 200 µmol/L, $\Theta_F * \Theta_F^{TOTB1}$ (1 DF)	-17.02	$\Theta_F = 0.193$	0.10	0.29	< 0.001
			$\Theta_F^{TOTB1} = 1.81$	1.59	2.03	
	$\Theta_F * (BSA/0.49)$	-27.286	$\Theta_F = 0.183$	0.12	0.24	N.A. [c]

[a] Reduction in the OBJF in comparison to the basic model (BASE 1). A ΔOBJF ≥ 7.9 and 10.6 is statistically significant ($p < 0.005$) for 1 and 2 degrees of freedom (DF), respectively. [b] CI = confidence interval. [c] N.A., statistical test inappropriate as it is a non-hierarchical model. [d] CL = 1.46 L/hr in BASE 1. [e] V = 39.1 L in BASE 1. [f] F = 0.197 in BASE 1.

The results of multivariate analyses with forward selection, incorporating the covariates with significant effects into BASE 2 one by one in decreasing order of reduction in OBJF are presented in Table 11. In the presence of the effect of WT on F, BSA and HT no longer had a significant effect on F. However, the effects of AGE on CL, TOTBIL on F and BSA on V remained significant. In the subsequent models, the effects of AGE on F, WT and HCT on V were no longer significant. Thus, a model incorporating the effects of AGE on CL, BSA on V, WT and TOTBIL on F was selected as BASE 3.

Backward elimination from BASE 3 showed that the effects of AGE on CL, BSA on V, body WT and TOTBIL on F remained statistically significant; the model incorporating these factors was declared the final model for Tac population PK. An adequate correlation between predicted and observed whole blood Tac concentrations was observed in the final model as shown in Figure 4.

Model evaluation

Mean prediction error (ME) between measured and predicted blood Tac concentrations in the validation group (n = 4) of paediatric liver transplant patients was 1.4 ng/mL (95 % CI: -0.025, 2.81). Thus there was a statistically insignificant bias between the measured and predicted blood Tac concentrations. The root mean square error (RMSE) associated with the validation group was 6.7 ng/mL (95 % CI: 5.58, 7.68), which is similar to the intrapatient s.d. for the population group of 5.8 ng/mL. The scatterplot of weighted residual versus predicted whole blood Tac concentration (Figure 5) showed that the weighted residuals were

Table 11. Summary of multivariate analyses with forward selection.

Effect	ΔOBJF [a]	Improvement [b]
WT on F (BASE 2)	-	-
BSA on F	2.728	No
HT on F	-1.283	No
AGE on CL	-30.037	Yes
TOTBIL on F	-8.294	Yes
BSA on V	-19.892	Yes [c]
AGE on F	4.109	No
WT on V	6.787	No
HCT on V	-3.297	No

[a] Change in OBJF in comparison to preceding model with an advantage.
[b] Advantage on preceding model with significant effect (based on ΔOBJF and 95% confidence interval).
[c] This model which incorporates the effects of AGE on CL, BSA on V, WT and TOTBIL on F was selected as BASE 3.

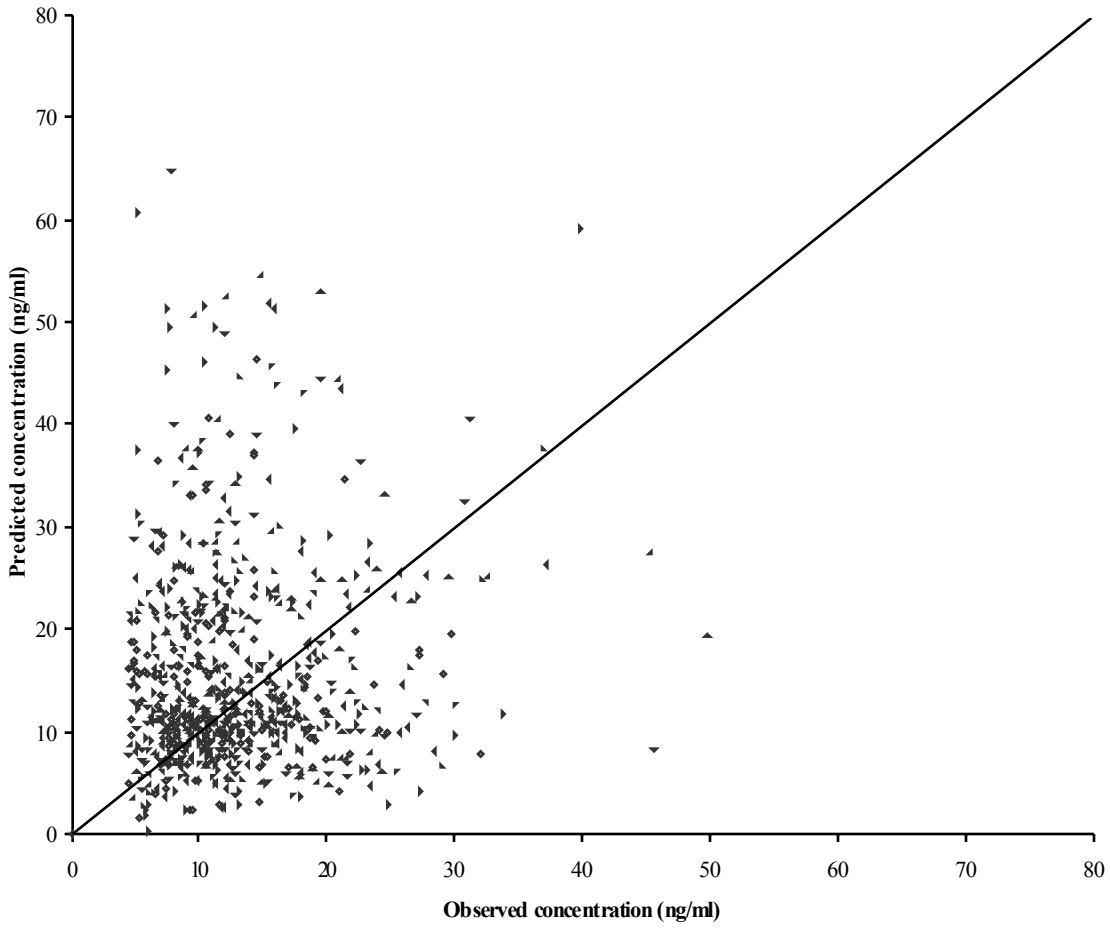


Figure 4. Scatterplot of predicted versus observed Tac whole blood concentration in the population (index) group (n = 16 patients) of the final model.

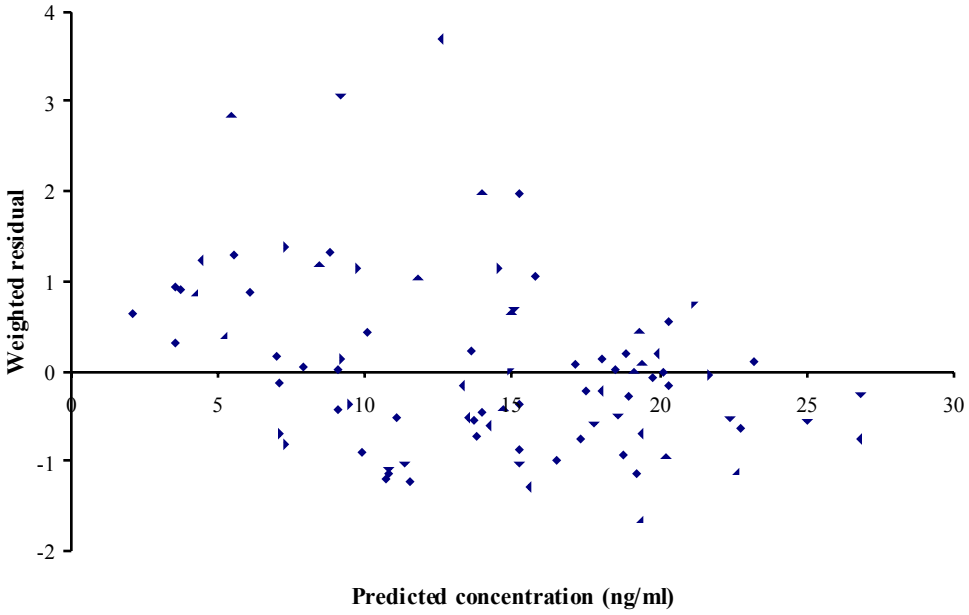


Figure 5. Predictive performance of the final model (n = 4 patients). Scatterplot of weighted residual versus predicted Tac whole blood concentration.

randomly distributed and mostly lay within ± 2 units of the null ordinate of perfect agreement. Specific examples of the predictive capability of the final optimal model are shown for two representative individual patients in the validation dataset in Figure 6, which shows the time-course of measured and posthoc predicted whole blood Tac concentrations.

Final population PK model

The final computed population parameter estimates, the interpatient and residual variability and the precision of the estimates obtained by fitting the full dataset are presented in Table 12. For a hypothetical patient with population median values of AGE, BSA and body WT (i.e. 2.25 years old, 0.49 m^2 and 11.4 kg), the model-predicted CL, V and F would be 1.46 L/hr, 39.1 L and 0.197, respectively. The F would increase by 61 % to 0.317 if the TOTBIL was ≥ 200 μmol/L. The model for CL found that CL changed by 34 % for every 1 year above and below 2.25 year (the median AGE), resulting in an estimated range of 0.88 – 7.23 L/hr across the AGE range of 1.07 – 13.9 year. The model for V indicated that V changed by 46 % for every 0.1 m^2 above and below 0.49 m^2 (the median BSA), which resulted in an estimated range of 15.9 – 96.3 L across the BSA range of 0.36 – 0.81 m^2. The model for F showed that it changed by 0.09 % for every 1 kg above and below 11.4 kg (the median body WT) giving an estimated range of 0.118 – 0.356 across the body WT range of 6.9 – 20.5 kg. Profile of age-normalised CL (predicted by the population model) vs AGE of patient is shown in Figure 7.

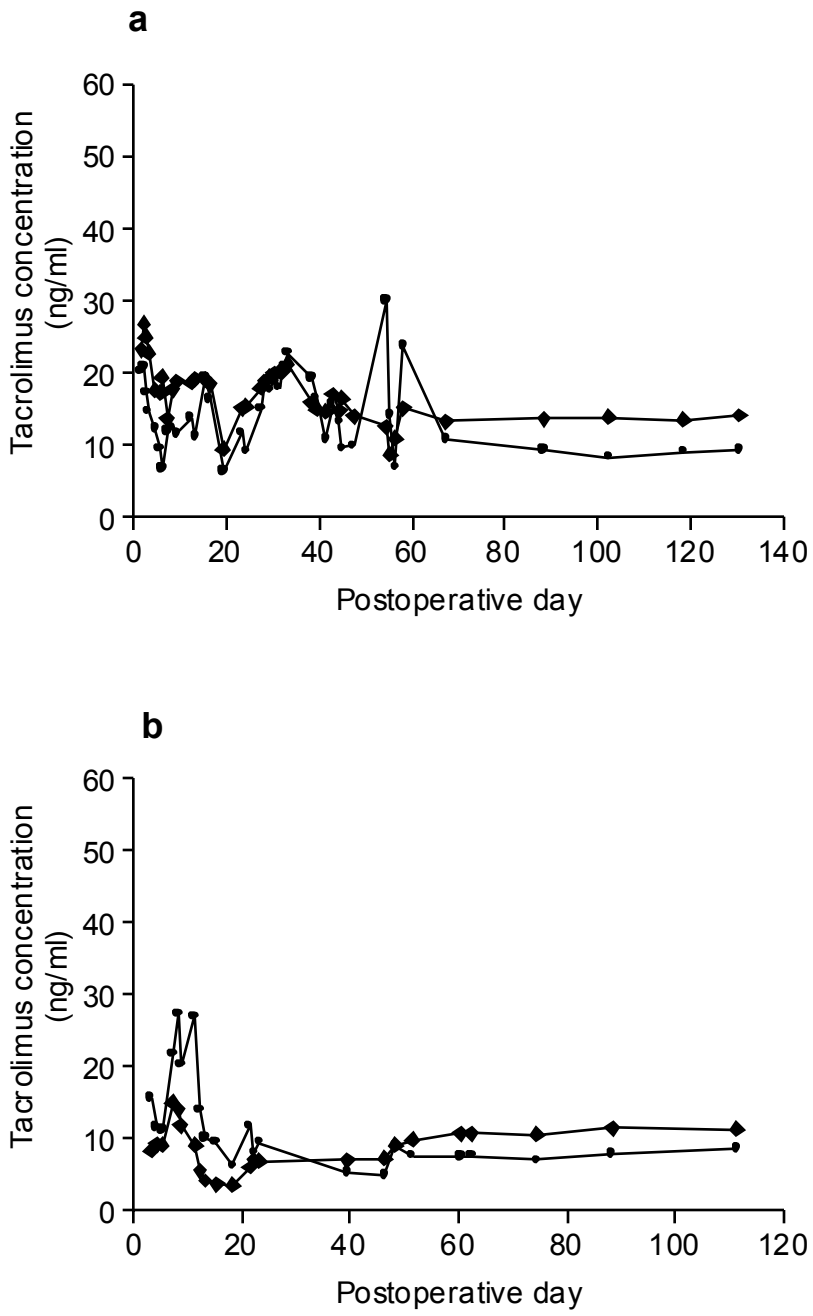


Figure 6. Longitudinal assessment of the predictive performance of the final population model in 2 representative patients from the validation dataset: (a) 1 year-old male (b) 1 year-old female. (●) observed and (■) model-predicted whole blood Tac concentration.

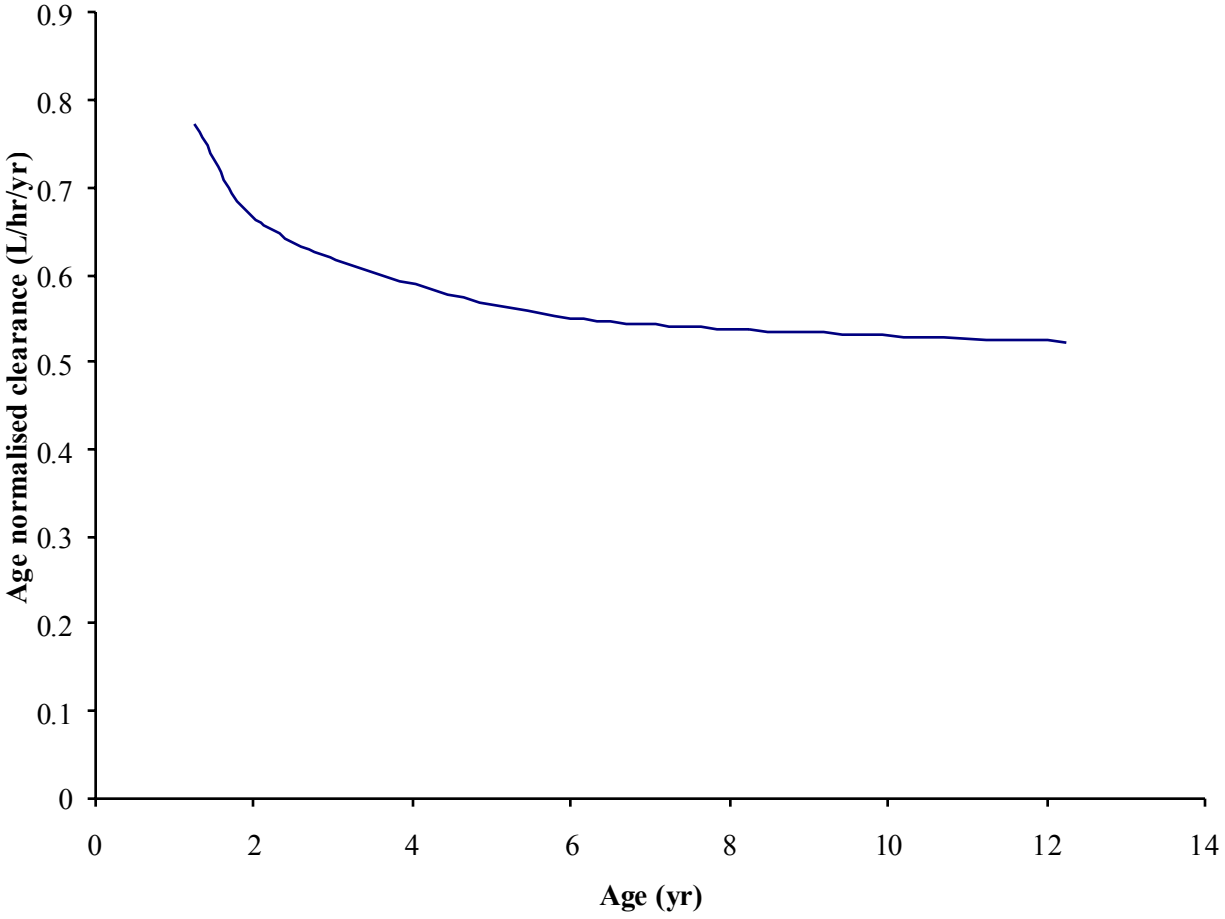


Figure 7. Profile of age-normalised CL (predicted by the population model) vs AGE of patient.

Table 12. Postliver transplantation population PK of Tac after IV and oral administration in Asian paediatric patients.

Parameter	Symbol	Units	Estimated value	Precision of estimation Std. error	CV (%)
CL	Θ_{CL}	L/hr	1.46	0.174	11.9
V	Θ_{V}	L	39.1	8.36	21.4
F	Θ_{F}		0.197	0.0263	13.4
Factor for AGE on CL	Θ_{CL}^{AGE1}	yr^{-1}	0.339	0.0675	19.9
Factor for BSA on V	Θ_{V}^{BSA1}	m^{-2}	4.57	0.983	21.5
Factor for body WT on F	Θ_{F}^{WT1}	kg^{-1}	0.0887	0.0228	25.7
Factor for TOTBIL ≥ 200 μmol/L on F	Θ_{F}^{TOTBIL}		1.61	0.0991	6.16
Interpatient variance of CL_j about CL_{TV}	ω^2_{CL}		0.112	0.0446	39.8
Interpatient variance of V_j about V_{TV}	ω^2_{V}		0.109	0.0756	69.4
Interpatient variance of F_j about F_{TV}	ω^2_{F}		0.0579	0.0451	77.9
CV of CL_j about CL_{TV}	CV_{CL}	%	33.5		
CV of V_j about V_{TV}	CV_{V}	%	33.0		
CV of F_j about F_{TV}	CV_{F}	%	24.1		
Intrapatient variance of C_{ij} about $C_{pred,\,ij}$	σ^2		0.0000335	0.00000561	16.7
Standard deviation of C_{ij} about $C_{pred,\,ij}$	σ	ng/mL	5.79		

Structural models:

$$TVCL = \Theta_{CL} * [\,1 + \Theta_{CL}^{AGE1} * (AGE - 2.25)\,]$$

$$TVV = \Theta_{V} * [\,1 + \Theta_{V}^{BSA1} * (BSA - 0.49)\,]$$

$$TVF = \Theta_{F} * [\,1 + \Theta_{F}^{WT1} * (WT - 11.4)\,] * [(1 - Y) + Y * \Theta_{F}^{TOTBIL}\,]$$

Random effects models:

$$CL_j = TVCL * (\,1 + \eta_{j,CL}\,)$$

$$V_j = TVV * (\,1 + \eta_{j,V}\,)$$

$$F_j = TVF * (\,1 + \eta_{j,F}\,)$$

$$C_{ij} = C_{pred,\,ij} + \epsilon_{ij}$$

where, AGE = age in year; BSA = body surface area in m^2; WT = body weight in kg; TVCL = typical population value for CL; TVV = typical population value for V; TVF = typical population value for F; and the value of Y is 0 for total bilirubin < 200 μmol/L. The Y value for total bilirubin ≥ 200 μmol/L is 1.

The interpatient variability (CV %) for the population PK parameters of CL, V and F was large and ranged from 24-34 % (Table 12). The intrapatient s.d. was 5.79 ng/mL, which translates to a CV % of 45.6 % at the mean blood concentration of Tac (12.7 ng/mL) measured in the population dataset. The imprecision (calculated by dividing the standard error of each parameter by its value and expressed as a percentage) in estimating the random effect parameter estimates was greater than that of the fixed effect parameter estimates.

3.4. Discussion

In this study, the PK of Tac were investigated in Asian paediatric liver transplant patients by a population modeling approach. This is particularly suitable as ethical and logistical restrictions involved in studying children prohibit extensive blood sampling, compared with traditional PK studies (Kauffman and Kearns, 1992). Large physiological and maturational changes occur in paediatric patients as they are in a rapid stage of development, thus producing potentially greater impact on the PK and therefore may be at greater risk from inadequate dosing. However, it must be noted that while this approach allows the development of a population model and the identification of potential covariates, the results needs to be verified in prospective PK studies.

Tac is extensively metabolised in the liver and undergoes biliary excretion (Venkataramanan *et al.*, 1995). In this study, part of the interindividual variability in CL was explained by differences in the AGE of the patient. The interindividual variability of

CL in BASE 1 was 53.9 %; however, on considering the patient's AGE, this variability was reduced to 33.5 %. From the general principles of developmental pharmacology (Yaffe and Aranda, 1992; Radde and MacLeod, 1993), it would be expected that a highly lipophilic drug, such as Tac, eliminated primarily by biotransformation, will show age-related differences in its PK. In addition, as a result of dosage problems and rapid changes in clinical condition, growth and maturation would cause further intraindividual differences.

The mean population CL value of Tac found in this study was 0.125 L/hr/kg (obtained by normalising the mean population CL value of 1.46 L/hr by the population mean body weight of 11.65 kg for the 20 patients), which closely agreed with the 0.138 L/hr/kg obtained by the traditional approach used in one study on paediatric patients (Wallemacq *et al.*, 1998). This is about twice the mean CL of 0.0541 L/hr/kg (Jusko *et al.*, 1995a) and 0.0528 L/hr/kg (Lee *et al.*, 1993) previously estimated in adult liver transplant recipients and could account for the findings that paediatric patients require higher doses of Tac on a mg/kg basis than adults (Jain *et al.*, 1991; McDiarmid *et al.*, 1993).

The dependence of Tac CL normalised for age with age (Figure 7) indicates a decrease in age-normalised CL with increasing age. The physiological basis for age-related decrease in age-normalised CL is unknown but may be related to changes in hepatic metabolic function with age. Studies of alterations in rates of hepatic metabolism for a wide variety of pharmacologic agents as a function of increasing age have indicated

that, whereas nonmicrosomal metabolism and Phase II conjugation processes are minimally affected, the activity of the microsomal mixed function oxidase (MFO) system decreases progressively with increasing age (Munson *et al.*, 1996). Thus the hepatic metabolism of Tac decreases with increasing age as Tac can undergo biotransformation both by the MFO and conjugation (Venkataramanan *et al.*, 1995). Many drugs that are biotransformed by oxidation are more rapidly metabolised during childhood than in adulthood, particularly if the drug CL is normalised to body WT. Examples of drugs with higher rates of biotransformation during childhood include theophylline, phenobarbital and phenytoin (Heimann and Gladtke, 1977; Blain *et al.*, 1981; Chiba, 1991). The activities of many components of the mono-oxygenase system are higher in children under 12 years of age and to a lesser extent during puberty, than in adults (Radde and MacLeod, 1993).

As reported in a previous paediatrics study (Yasuhara *et al.*, 1995), the CL of Tac correlated with the number of postoperative days (POD) and body size (expressed as body weight raised by to the power of 0.29). The correlation with POD was attributed to change in hepatic function with POD, but in the present study no such correlation was found. A previous population PK analysis of Tac in adults (Mekki and Lee, 1994) showed that mild to moderate hepatic dysfunction did not affect the CL of Tac, which was also found in our paediatric patients. However, it should be noted that only one patient in our population dataset suffered from severe hepatic dysfunction over a sustained period.

Indices of renal function (serum CREA and serum UREA), GEN, BSA, HCT and ALB had no significant effect on the CL of Tac. The lack of effect of indices of renal function on CL is plausible since the renal CL of Tac accounts for less than 1 % of total systemic CL (Venkataramanan *et al.*, 1991).

Tac is a low-CL drug; the extraction ratio is equivalent to about 3 % of liver blood flow (Undre *et al.*, 1999). For a highly bound, low-extraction ratio drug like Tac, CL would be affected by changes in HCT and plasma protein binding. Indeed, trough whole-blood concentrations of Tac after renal transplantation correlate with HCT and ALB during the first weeks of treatment and its relative CL was negatively correlated with HCT and ALB (Undre and Schäfer, 1998). The lack of significant effect of HCT and ALB on CL in the current study could be attributed to the limited range of HCT and ALB concentrations in our patients.

Part of the interindividual variability in the V of Tac was explained by differences in the BSA of the patient. The interindividual variability of V in BASE 1 was 61.2 %; however, on considering the patient's BSA, this variability in V was reduced to 33.0 %. Therefore, BSA is a good indicator of the V in this group of paediatric patients. Body WT raised to a power of 0.29 is used as an indicator of body size to model the influence of body WT on V in a previous report in paediatric liver transplant patients (Yasuhara *et al.*, 1995). Although both the body WT and BSA had significant effects on V during the univariate analysis in this study, BSA was found to be a better predictor of V than body WT. This may be because the body WT measured in these patients is not an accurate

reflection of lean body mass as most of the time there is an accumulation of extracellular fluid (in the form of ascites fluid) in these patients. Thus, the body WT in this group of patients is variable and is susceptible to fluctuating values depending on the clinical condition of the patient. Thus, the incorporation of body HT with body WT in the form of BSA gives a better indicator of body size and hence predictor of V, as HT is not susceptible to wide fluctuations in values.

The mean population V value of Tac found in this study was 3.36 L/kg (obtained by normalising the mean population V value of 39.1 L by the population mean body WT of 11.65 kg for the 20 patients). This is in reasonable agreement with the V values of 2.6 L/kg and 41.41 L determined in a traditional PK study in paediatric patients (Wallemacq *et al.*, 1998) and a population PK study (Yasuhara *et al.*, 1995), respectively. However, this is about three times the mean value of V of 0.906 L/kg reported in the adult liver transplant recipients (Jusko *et al.*, 1995a). The V decreases with increasing age, which may be due to age-related changes in binding substances such as HCT with age (Natha and Oski, 1987), which would affect the distribution of the drug within the body. This is because Tac is extensively distributed into red blood cells, and the whole blood to plasma ratio ranges are greater than 30-10 over low to high plasma concentrations (Jusko and D'Ambrosio, 1991; Backman *et al.*, 1994). Therefore, changes in HCT would alter the distribution of Tac between blood and fat since it is a lipophilic compound. The highest normal HCT value (0.61 L/L) is seen in newborns, followed by a gradual decrease to reach a nadir at 2 months of age. The HCT then increases until normal adult values (about 0.45 L/L) are reached at 14 years of age. Thus, the increase in the HCT from 2

months to 14 years of age could decrease the partitioning of Tac into fat, thereby decreasing its distribution. This hypothesis is supported by the finding that HCT was a significant covariate affecting V during the univariate analysis; an increase in HCT resulted in a decrease in V, via a power function. However, this model failed to be included in BASE 3 during subsequent multivariate analysis with forward addition. This does not mean that the HCT is not important; it simply reflects a lack of variability within the patient group that is not already accounted for by the effect of BSA on V. If a wider range of HCT had been present, it is likely that HCT would have had a significant influence on V.

GEN of the patient has no effect on V of Tac as shown in this study. Adult females have a higher proportion of body WT as fat than males, therefore GEN-related difference in V might be anticipated for Tac. However, all patients were pre-pubertal, which may explain the lack of GEN-related differences in body fat content in our patients.

Part of the interindividual variability in the F of Tac was explained by differences in the body WT and TOTBIL. The interindividual variability of F in BASE 1 was 35.2 %; however, on including the patient's body WT and TOTBIL, this variability is reduced to 24.1 % in the final population model. Thus the F of Tac is affected by two factors: the development and growth of the patient, and the liver function of the patient.

Since the extent of drug absorption is proportional to the area available for absorption, bowel length is a determinant of drug absorption, as reported for cyclosporine

(Whitington *et al.*, 1990). Hence the dependence of F on the development and growth of the paediatric patient is not surprising as the gastrointestinal tract undergoes considerable developmental changes during the first years of life (Motil, 1993). It has been found that the main factor contributing to increasing bowel length up to about 4 years of age is increasing body size (Seibert, 1980). Predictors of body size such as body WT, HT and surface area were all significant covariates affecting the F during the univariate analysis, but only body WT remained as a significant covariate during subsequent multivariate analysis. The discrepancy between the V and F models using different covariates as indicators of body size can be attributed to nondevelopmental factors affecting the bowel length such as surgical excision of part of the bowel during liver transplantation, or during Kansai portoenterostomy.

The liver function of the patient as indicated by the TOTBIL is found to affect the F of Tac. Paediatric patients with a TOTBIL ≥ 200 μmol/L had a 61 % greater F compared with those with lesser concentrations of bilirubin. This may be due to reduced first-pass effect in patients with hepatic dysfunction compared with patients with normal hepatic function resulting in higher F. This is supported by the finding in a study that a higher oral F of 36 % was noted in patients with moderate to severe liver impairment after infusion of Tac 0.15 mg/kg or an oral dose of 0.15 mg/kg (Jain *et al.*, 1990).

The mean population F calculated in the present study compared favourably with the value of 0.197 obtained in one study (Yasuhara *et al.*, 1995) using a population PK approach, and the value of 0.25 obtained in another study (Wallemacq *et al.*, 1998) in

paediatric patients using a traditional approach. Mean values of 0.25 and 0.238 were reported in adult liver transplant patients in a traditional PK study (Jusko *et al.*, 1995a), and in a population PK study (Mekki and Lee, 1994), respectively. Thus, Tac has a relatively low oral F both in paediatric and adult liver transplant patients.

The residual (unexplained) variability was quite large, reflecting perhaps large intraindividual variability in the PK, interoccasion variability, assay errors, sampling time errors and model misspecification. This variability may be reduced if a prospective study design is used which provides tighter control on some of the above factors.

In this study, the effects of covariates which have an influence on the PK parameters of Tac in paediatric liver transplant patients are due to a difference of covariates between patients and the change within a patient for that covariate. This is because in many patients, the data were collected for a duration of several months, which will result in changes of covariate values within a patient. One limitation of the current study is that only a small number of patients (n = 20) was used for analysis. The ability to encounter and quantify many covariates is quite limited in such a small group. However, this was the number of paediatric patients that were currently available at the time of this analysis, as there were 22 paediatric liver transplantations performed during the period 1995 to May 1999 (two retransplants) (Wai *et al.*, 2000) at National University Hospital, Singapore.

Therefore the purpose of this population analysis is mainly hypothesis-generating, ie. producing hypothesis of the effects of covariates on the PK parameters. This population model can be tested and refined using data from future prospective studies with more subjects, and this may have the potential to yield appropriate dosing recommendations.

POPULATION PHARMACOKINETICS OF TACROLIMUS IN ASIAN ADULT AND PAEDIATRIC LIVER TRANSPLANT PATIENTS

4.1. Study Aims

The objective of the study is to prospectively describe the population PK of Tac following oral administration in Asian adult and paediatric liver transplant patients, and to identify and quantify the influence of patient characteristics on the PK of Tac.

The previous study in this thesis has focused on the same aspects but in a sub-population i.e. paediatric patients. A hypothesis regarding the effect of HCT on the V of Tac was made based on the findings in the study. It is of interest to verify this hypothesis on a wider range of HCT. This is only possible if adult patients are included in the same study to allow the effect to be investigated and quantified. In general, a population model built from a more heterogenous population allows an easier determination of the effect of covariates on the PK parameters because a wider range of values for the covariates can be encountered.

4.2. Methods

4.2.1. Chemicals and materials

The following drugs and chemicals were kindly provided by or obtained from the sources indicated: Tac (FK-506) (Fujisawa Pharmaceuticals Co., Osaka, Japan), ascomycin (BioChemika, Fluka Chemie AG and RdH Laborchemikalien GmbH & Co. KG), HPLC-grade solvents such as acetonitrile and methanol used in extraction or

analytical procedures (Fisher Chemical, Fisher Scientific, Fair Lawn, New Jersey), ammonium formate (Analar grade) (BDH Chemicals, Poole, U.K.), acetic acid (ACS grade) (Sigma Chemical, St. Louis, MO), zinc sulphate (Merck, Darmstadt, Germany). Deionized (18 mΩ) water (Milli-Q water system; Millipore Inc., Bedford, MA) was used.

Ascomycin is a structural analogue of Tac. The chemical structure of ascomycin is shown in Figure 8.

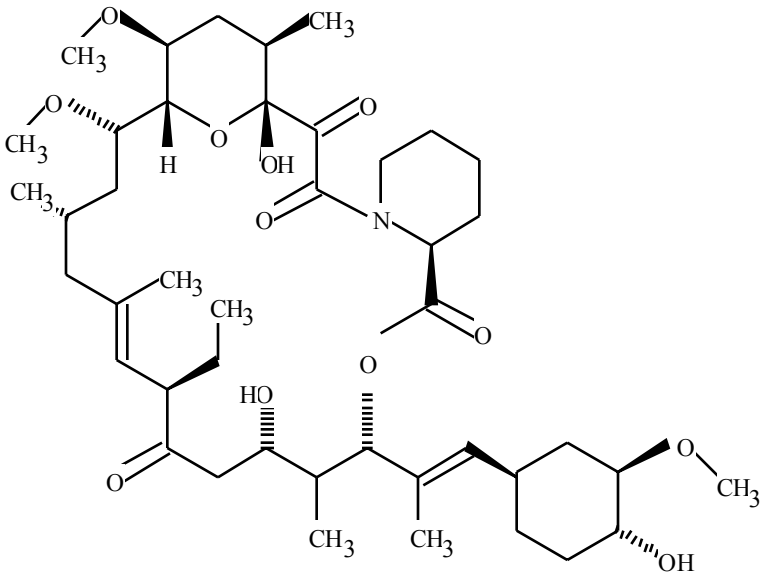


Figure 8. Chemical structure of ascomycin.

4.2.2. Preparation of standard, internal standard and quality controls

Two different Tac stock solutions of the same concentration were prepared separately, one of which was used for the preparation of calibration standards and the second for quality control samples. Tac stock solution I (1 mg/mL) was prepared fresh

with the weighing of Tac and dissolution in acetonitrile. Stock solution I was diluted 100-fold with acetonitrile to yield stock solution II. Stock solution II (10 µg/mL) was diluted 2000-, 500-, 100-, 50-, 20-, 10- and 5-fold with acetonitrile to produce 5, 20, 100, 200, 500, 1000 and 2000 ng/mL working stocks of Tac, respectively. A 100 µL aliquot of each working stock was added to 1.90 mL of blank human whole blood to prepare whole blood calibration standards. A 100 µL aliquot of the 5, 20, 100, 200, and 500 ng/mL working stock was added to 1.90 mL of blank human plasma to prepare plasma calibration standards.

Ascomycin stock solution (1 mg/mL) was prepared in acetonitrile. This solution was diluted with acetonitrile to prepare a 2500 ng/mL working internal standard solution. Addition of 10 µL aliquots of the working solution to 1 mL human whole blood or plasma gave a concentration of 25 ng/mL.

Tac stock solution II (10 µg/mL) was diluted 2000-, 333.3-, 33.3- and 6.7-fold with acetonitrile to produce 5, 30, 300, and 1500 ng/mL working stocks of Tac, respectively. Whole blood quality controls were prepared by diluting 5.0 mL aliquots of these working stock solutions to 100 mL with blank human whole blood. Stock solution II (10 µg/mL) was diluted 2000-, 333.3-, 66.7- and 25-fold with acetonitrile to produce 5, 10, 150, and 400 ng/mL working stocks of Tac, respectively. Plasma quality controls were prepared by diluting 5.0 mL aliquots of these working stock solutions to 100 mL with blank human plasma.

4.2.3. Calibration and validation

The determination of Tac was based on the internal standard method. The calibration curve was generated by plotting the peak area ratio of Tac to the internal standard, ascomycin, against the spiked concentration of Tac. A seven-point calibration curve (triplicate injections) was created for the range 0.25 – 100 ng/mL for the quantitation of Tac in human whole blood. A five-point calibration curve (triplicate injections) was created for the range 0.25 – 25 ng/mL for the quantitation of Tac in human plasma. Calibration curves for both whole blood and plasma matrices were evaluated by ordinary least-squares regression.

Assay performance was assessed by precision and accuracy of the standard concentrations used for the quality control samples. Precision was expressed as the percent CV at each concentration. Precision was calculated after the analysis of 6 replicates on the same analytical run (intra-day) and after 3 repeated analyses on different days along different analytical runs (inter-day). Accuracy was calculated as the percentage of the added concentration multiplied by 100.

The extraction recoveries were determined from the whole blood and plasma samples used for the precision study. The mass spectrometer responses of the extracted samples were compared with the response after injection of respective amounts of Tac or ascomycin solutions directly on the analytical column.

4.2.4. Sample preparation

The sample preparation method for Tac in human whole blood and plasma is adapted from a method for therapeutic monitoring of Tac in blood, with modifications (Lensmeyer and Poquette, 2001). The modifications involve the change from semi-automated solid phase extraction to a fully manual procedure and the change from the use of styrene-divinylbenzene (SDB-XC) disk cartridge to a C_{18} reversed-phase disk cartridge.

Whole blood samples

1 mL of whole blood was spiked with 10 µL of ascomycin working standard solution. 2 mL of a 0.3M zinc sulphate:methanol (40:60 v/v) solution (protein precipitating solution) was added to the sample to precipitate the proteins. The sample was then vortexed, centrifuged at 20,000g for 10 minutes and the supernatant subjected to solid-phase extraction by use of a C_{18} 7 mm/3 mL reversed-phase extraction disk cartridges (3M Empore™, Fisher Scientific, Fair Lawn, NJ) previously preconditioned with methanol and water on a vacuum manifold (Varian Sample Preparation Products, Harbor City, CA). Tac was eluted with 3 mL of acetonitrile:water (90:10 v/v) and the eluant dried using a Savant SpeedVac® concentrator (Savant, Holbrook, NY). The dried sample was reconstituted in 60 µL of acetonitrile:water (90:10 v/v) and 20 µL was injected using a 25 µL micro-syringe (Hamilton Co., Reno, Nevada).

Plasma samples

The same procedures were used for preparing the plasma samples except that 2 mL of samples, 20 µL of internal standard solution and 3 mL of protein precipitating solution were used.

4.2.5. Blood sampling

Venous blood samples (6.5 mL) were taken from an arm vein just before a dose and at specific time points after an oral dose of tacrolimus. Blood samples were divided over two EDTA Vacutainer® Plus blood collection tubes (Becton Dickinson, Franklin Lakes, NJ). One 1-mL whole blood sample was stored at –80ºC until the time of drug assay. The other blood sample was incubated at 37ºC in a water bath for 2 hours before centrifuging for 10 minutes at 3000 rpm at the same temperature. Separated plasma was aspirated with a disposable pipette and transferred to a 2.5 mL plastic tube. The plasma sample was also stored at –80ºC until assayed for Tac.

4.2.6. Bioanalytical assay

Whole blood and plasma concentrations of Tac were determined using an electrospray high-performance liquid chromatographic/mass spectrophotometric/mass spectrophotometric (HPLC/MS/MS) assay. This assay was modified from an assay published by Taylor *et al* (Taylor *et al.*, 1996). The modifications include the change of

the organic solvent used in liquid chromatography from methanol to acetonitrile, the flow rate of the mobile phase decreased from 0.1 ml/min to 0.05 ml/min and the column was changed from a C_4 reversed phase 30 mm × 2.1 mm column to a C_{18} reversed phase 50 mm × 1.0 mm column.

The HPLC/MS/MS system consisted of a Shimadzu (Kyoto, Japan) LC-10 AD microbore HPLC with a binary pump and 15 μL gradient mixing chamber, interfaced to a Perkin Elmer (PE-Sciex, Thornhill, Toronto, Canada) API 300 triple quadrupole mass spectrometer operated in the multiple-reaction monitoring (MRM) mode. A 50 mm × 1.0 mm, 3-μm Luna C18(2) reversed-phase column (Phenomenex Inc., Torrance, California) was installed in the instrument and was operated at 55ºC using a column heater (Fiatron CH-30, Milwaukee, Wisconsin). The isocratic mobile phase was composed of 75 % acetonitrile/25 % ammonium formate buffer (2 mM). The liquid chromatography separation was performed at 50°C with a flow rate of 0.05 mL/min. The total run time was 7 minutes.

The ion source (heated electrospray) used a turbo ionspray gas flowing at 6 L/min at 325°C. Nitrogen was used as the collision gas and the collision gas thickness was 1.4×10^{14} molecules/cm^2. Ionization was achieved in the positive mode using the following parameters: an ionization voltage of 4600 V and a ring voltage of 320 V; a collision energy of 31 V for the ammonium adduct ions and an orifice voltage of 31 V. The first quadrupole was set to select the ammonium adducts $[M+NH_4]^+$ of Tac (*m/z* 821.1) and ascomycin (*m/z* 809.6). The second quadrupole was used as a collision

chamber, and the third quadrupole was then used to select the characteristic product ions of Tac (*m/z* 576.1) and ascomycin (*m/z* 756.6). The retention times for Tac and ascomycin were 3.5 min. Following HPLC separation, the peak area corresponding to the MRM reaction (dwell time 150 ms) for Tac was measured relative to that of the MRM reaction (dwell time 150 ms) of the internal standard, ascomycin.

Peak area ratios obtained from MRM of the mass transitions for Tac (*m/z* 821.1 → 576.1) and ascomycin (*m/z* 809.6 → 756.6) were used to calculate the concentrations of Tac by use of a calibration curve. This was generated from the analysis of a blank blood sample spiked with various amounts of Tac and fixed amounts of internal standard.

4.2.7. Data analysis

The data analysis was carried out using a stepwise approach, according to a general approach suggested by Ette and Ludden (Ette and Ludden, 1995) with modifications: (1) determination of a basic PK model using the NONMEM program and obtaining the Bayesian individual parameter estimates, (2) selection of covariates using generalized additive modeling (GAM) and tree-based modeling (TBM), (3) final NONMEM modeling to determine the population PK model, and (4) evaluation of final population PK model using case-deletion diagnostics.

Step 1: Determination of basic PK model

Nonlinear mixed-effect models were used to characterize Tac population PK parameters from the whole blood concentration-time data using the NONMEM (version V; level 1.1; GloboMax LLC, Hanover, Md) with double precision. The first-order estimation method was used to derive population mean PK parameters, the intersubject variability (η) in these parameters, and residual variability between observed and predicted concentrations ($\in$). This model accounts for population parameter variability (between and within subjects) and residual variability (random effects), as well as parameter differences predicted by covariates (fixed effects).

One and two-compartment PK models with first-order absorption and elimination were tested. The one-compartment model was parameterized in terms of apparent CL (CL/F), apparent V (V/F) and k_a. The two-compartment model was parameterized in terms of CL/F, volume of central compartment, intercompartmental CL, volume of peripheral compartment and k_a.

Interindividual variability in CL/F was modeled using an exponential error model (Boeckmann *et al.*, 1992) as shown in the equation below.

$$(CL/F)_j = TV(CL/F)*(\exp \eta_{j,\,CL/F})$$

where CL/F_j is the hypothetical true total body CL/F for the jth individual as predicted by the regression model. TV(CL/F) is the typical population value of CL/F. The $\eta_{j, CL/F}$ is a random variable that represents the persistent difference between the jth individual's CL/F and that predicted by the regression model and is assumed to be independent and normally distributed with zero mean and variance ω^2. Interindividual variability in V/F is similarly modeled.

For residual variability, a series of error models was tested as follows:

$$C_{ij} = C_{pred, ij} + \in_{ij}$$

$$C_{ij} = C_{pred, ij} * (1 + \in_{ij})$$

$$C_{ij} = C_{pred, ij} * (1 + \in_{ij, 1}) + \in_{ij, 2}$$

where C_{ij} is the observed whole blood concentration and $C_{pred, ij}$ is the corresponding model predicted value for the jth individual. $\in_{ij, 1}$ and $\in_{ij, 2}$ are random terms that are assumed to have 0 mean and variances σ_1^2 and σ_2^2, respectively. The variances ω^2 and σ^2 were estimated as components of the population model.

With the fixed and random effects models chosen, empirical Bayes estimates of CL/F and V/F were obtained using the POSTHOC option within the NONMEM program.

Step 2: Selection of covariates

Stepwise GAM and TBM as implemented in Xpose3 (version 3.11) (Jonsson and Karlsson, 1999) running within the S-Plus (Version 2000; Insightful, Seattle, WA) program were performed on the empirical Bayesian parameter estimates for the selection of covariates which influence the PK parameters.

In the GAM analysis (Hastie and Tibshirani, 1990), the individual Bayes parameter estimates together with the covariates were entered in the analysis. At each step, individual covariates are added, deleted, or replaced in the regression model using Akaike's information criterion (AIC) (Akaike, 1976) until a final model is obtained. The final model indicated a set of candidate covariates and its relationship i.e. linear or nonlinear, with each PK parameter. The GAM assumes the following general functional form:

$$P_i = \alpha + f_1(z_{i1}) + f_2(z_{i2}) + \ldots. + f_n(z_{in})$$

where p_i is the ith individual's parameter value, α is the intercept, z_{in} is the ith individual's value of the nth covariate and the f ( )s are linear or spline functions that relate the influence of the covariates to the parameter. The stepwise search is carried out according to a defined hierarchy (one for each covariate) of possible functional relationships, which for this analysis is: the covariate is not included in the model, the covariate is included in a linear fashion and the covariate is included in a nonlinear

fashion. The GAM approach provides for straightforward interpretation of results by assuming an additive structure. It also allows the contributions of various covariates to be displayed graphically.

Bootstrap in conjunction with GAM is run to assess the importance of the covariates and to give information about how covariates interact with respect to inclusion/exclusion from the covariate model. In bootstrap (Efron and Tibsgirani, 1993) of the GAM, the GAM is run a large number of times on bootstrap realizations of the original data set. The bootstrapped data sets are constructed by random sample with replacement from the original data set and the GAM is then run on each of them. The number of bootstrap iterations made in this study was 150.

TBM is an exploratory technique for uncovering structure in data, and assessing the adequacy of linear models (Breiman *et al.*, 1984). A regression tree is built through a process known as binary recursive partitioning. The data are split into two parts, along with any of the X predictors (covariates), so that the resulting groups are most homogeneous with respect to the response. Specifically, all splitting points are examined along all predictors, and the one that produces the smallest total within-group variance in the two groups is chosen. The split at a node is that split on the X variables which most successfully separates the high response values from the low ones. The data are then split into two parts, and the process is repeated on each part. At each stage, all split points along all predictors are considered, so that the predictor can be used for splitting more than once. The splitting process can be terminated when no further splits can be found to

significantly improve the homogeneity of the subgroups. At this point, an unpruned tree has been "grown". An unpruned tree usually includes too many split points compared to a situation where statistical significance check had been applied to each split.

Cross-validation is used to determine the appropriate tree size. First, the data set is divided into x number of groups, second, x number of trees are grown, each with one of the x groups omitted from the growth process and third, the fitted trees are used to predict the group of data not used to grow the tree. The improvement in the fit (measured by the deviance), at each node of the tree, is averaged over the x groups and the results are plotted. The improvement in fit will only continue up to a certain point after which the deviance will increase, i.e. the plot of the averaged deviance vs tree size will often exhibit a minimum at some point, which is the suggested optimal tree size.

Step 3: Population model building using NONMEM

Each covariate screened as influential by the graphic explorations was entered into the population PK model derived in step 1. The continuous covariates were centered on their median values. In some cases, covariates not identified in step 2, yet bearing a logical relationship to a PK parameter, were also evaluated in the population model.

Depending on the nature of the covariate, a linear model for continuous variables or a step model for dichotomous variables was tested by stepwise addition to the basic

model as follows for TV(CL/F):

$$TV(CL/F) = \theta_1 + \theta_2 * (covariate_1 - median_1)...$$

$$TV(CL/F) = \theta_1 + \theta_3 * (covariate_2)\ldots$$

where the θ's are the parameters to be estimated. The covariate in the first equation is a continuous variable and the median is the corresponding median value of the population. The covariate in the second equation is a dichotomous variable. The covariates were entered in a similar manner for TV(V/F).

In the comparisons between hierarchical models, a likelihood ratio test was used. The ΔOBJF produced by the inclusion of a covariate is proportional to twice the negative log likelihood of the data and approximates a χ^2 distribution with degrees of freedom equal to the difference in the number of parameters between the two models. A ΔOBJF of > 3.8 ($P < 0.05$) is chosen to represent statistical significance for the addition of one fixed effect. If a covariate was statistically significant, it was kept in the model and a new covariate was added and tested. If not, then the covariate was dropped from the model and the effect of another covariate was evaluated (step-up approach). The tentative final model was further tested by eliminating each covariate one at a time to evaluate the ΔOBJF (step-down approach). Because of multiple comparisons, the level of significance for putting the covariate back in the model was set at ΔOBJF > 10.8 ($p < 0.001$, degree of freedom = 1). Only covariates that showed a significant contribution were preserved in the model.

The model building process was also guided by various goodness-of-fit plots, residual analyses, the standard error of the parameters and changes to estimates of ω's and σ resulting from changes to the model. Nonhierarchical models having equal numbers of parameters a ΔOBJF of 10 or more units were considered meaningful. The choice among models that exhibited similar overall goodness-of-fit characteristics was guided by parsimony and pragmatism. Parsimony is a principle that states that the simplest explanation that explains the greatest number of observations is preferred to more complex explanations. The parameters of the final model were re-estimated using the first-order conditional estimation (FOCE) in NONMEM.

Evaluation of population PK parameters

Case deletion diagnostics or cross-validation was used to detect influential individuals and to explore the robustness of the model. Cross-validation was performed by refitting the selected model with one patient excluded one-at-a-time and the data reanalyzed by NONMEM using the final model. The new parameter values obtained from the single case-deleted data set were compared qualitatively with those from all 31 individuals, which was the original number of patients in the dataset before single case deletion.

4.3. Results

4.3.1. HPLC/MS/MS assay

Under the HPLC-electrospray-MS/MS operating conditions, the predominant precursor for Tac and ascomycin were the ammoniated ion $[M+NH_4]^+$, *m/z* 821.1 and *m/z* 809.6, respectively. Collision-induced fragmentation of these precursor ions gave the predominant product ions for Tac and ascomycin of *m/z* 576.1 $[(M-210-H_2O)H]^+$ and *m/z* 756.6 $[(M-2H_2O)H]^+$, respectively. These mass transitions, *m/z* 821.1 → 576.1 and *m/z* 809.6 → 756.6, were utilized for MRM. The fragmentation pathway of Tac leading to the elimination of 210 Da is shown in Figure 9.

Typical MRM chromatograms of (A) a Tac whole blood standard (0.25 ng/mL), (B) a Tac whole blood standard (100.0 ng/mL), (C) whole blood sample containing 25 ng/mL ascomycin (D) a whole blood sample obtained from a liver transplant patient containing 40.3 ng/mL of Tac are illustrated in Figure 10.

4.3.2. Calibration and validation

Linear calibration curves were obtained with correlation coefficients, r greater than 0.99 (Figure 11); mean calibration curve parameters are reported in Table 13; mean peak area ratios±s.d. of the whole blood, and plasma calibration curves shown in Figure 11 are reported in Table 14.

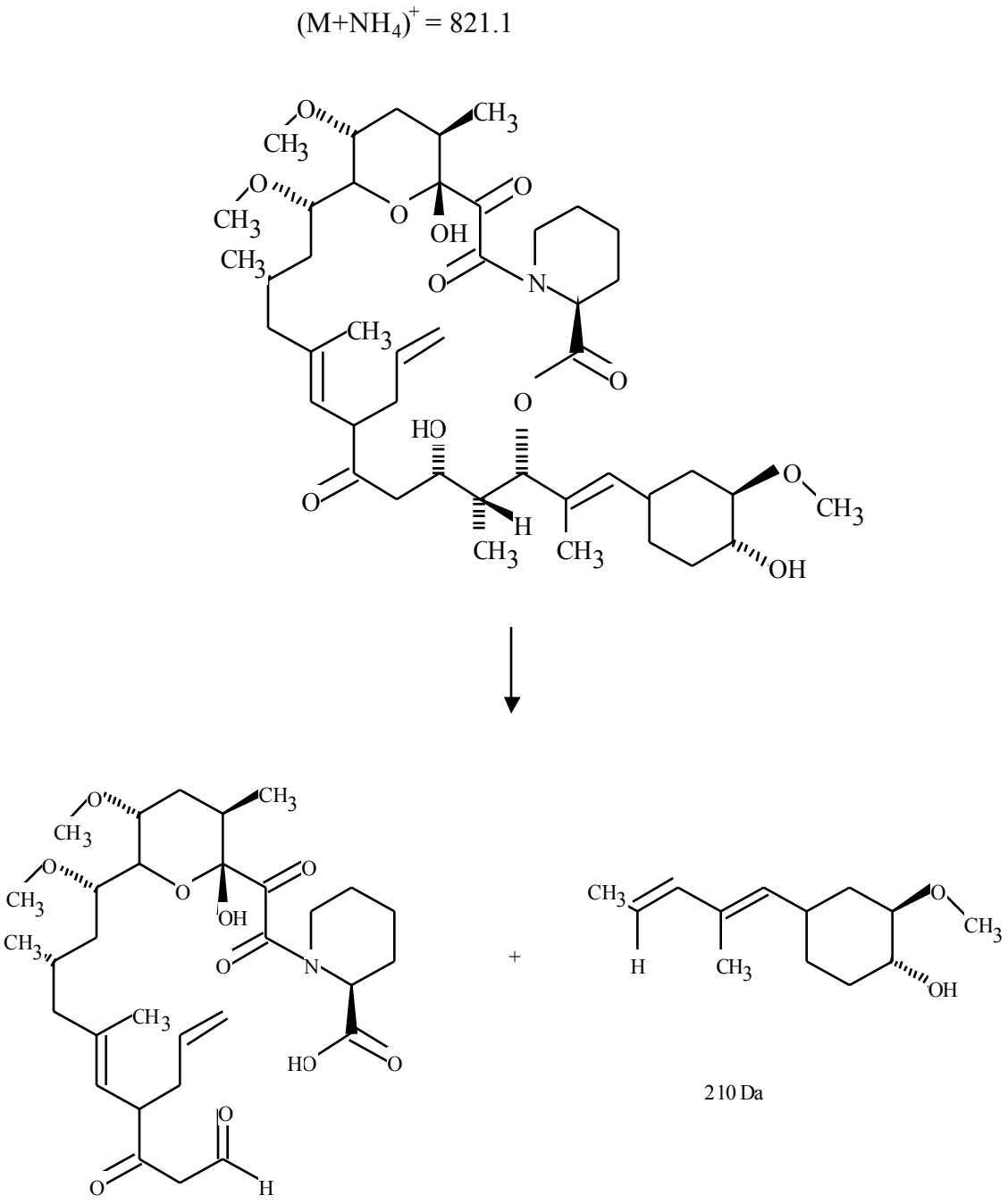
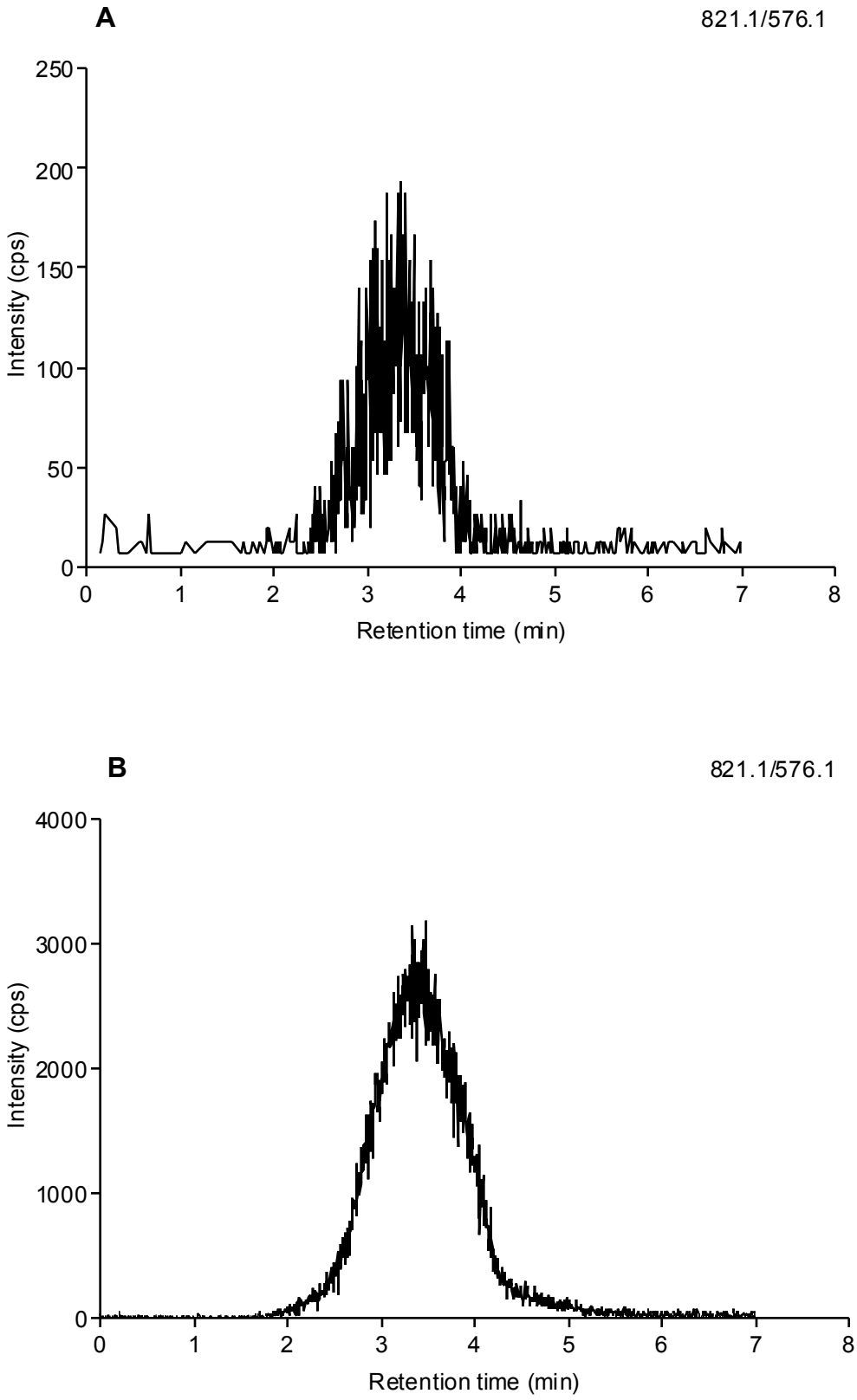


Figure 9. Fragmentation pathway of Tac leading to the loss of 210 Da.


A
821.1/576.1
250
200
150
100
50
0
Intensity (cps)
0
1
2
3
4
5
6
7
8
Retention time (min)
B
821.1/576.1
4000
3000
2000
1000
0
Intensity (cps)
0
1
2
3
4
5
6
7
8
Retention time (min)

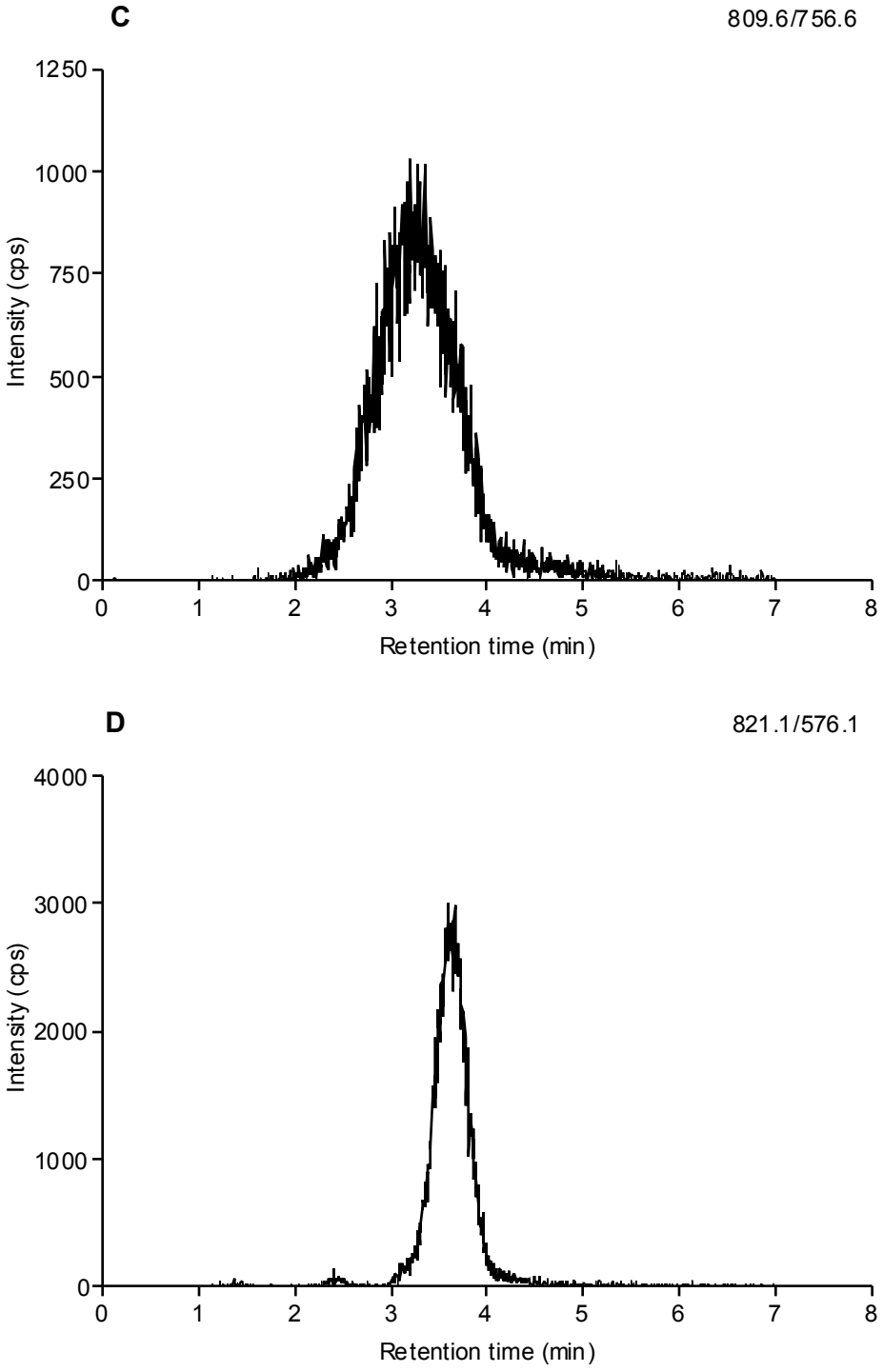


Figure 10. MRM chromatograms of (A) whole blood spiked with 0.25 ng/mL of Tac; (B) whole blood spiked with 100 ng/mL of Tac; (C) whole blood spiked with 25 ng/mL of ascomycin; and (D) clinical sample containing 40.3 ng/mL Tac.

The lower limit of quantification (LOQ) of Tac was assessed by pre-made whole blood / plasma samples. The LOQ was defined as the lowest concentration determined with an intra-day CV less than or equal to 20 % and with an accuracy of between 80 and 120 %, and was found to be 0.25 ng/mL for whole blood and plasma matrices (Table 15). Quality control samples, analysed in 6 replicates on 3 separate days, demonstrated acceptable inter-day variability of the system (Table 16). The absolute recovery is presented in Table 17. The recoveries of Tac and ascomycin from whole blood were between 59.1 and 67.1 % and from plasma were between 57.9 and 69.1 %.

	Y = mx + b		**Correlation coefficient (r ± SD)(%CV)**
	m (mean ± SD) (%CV)	**b (mean ± SD)(%CV)**	
Whole blood (n = 3)	0.0228 ± 0.0034 (14.9%)	0.002 ± 0.009[a] (450%)	0.9977 ± 0.0017 (0.17%)
Plasma (n = 3)	0.0267 ± 0.0028 (10.5%)	0.014 ± 0.025[b] (178.6%)	0.9955 ± 0.0033 (0.33%)

[a] Statistically insignificant from 0 (p=0.7725).

[b] Statistically insignificant from 0 (p=0.4288).

Table 13. Mean parameters of the calibration curves for Tac.

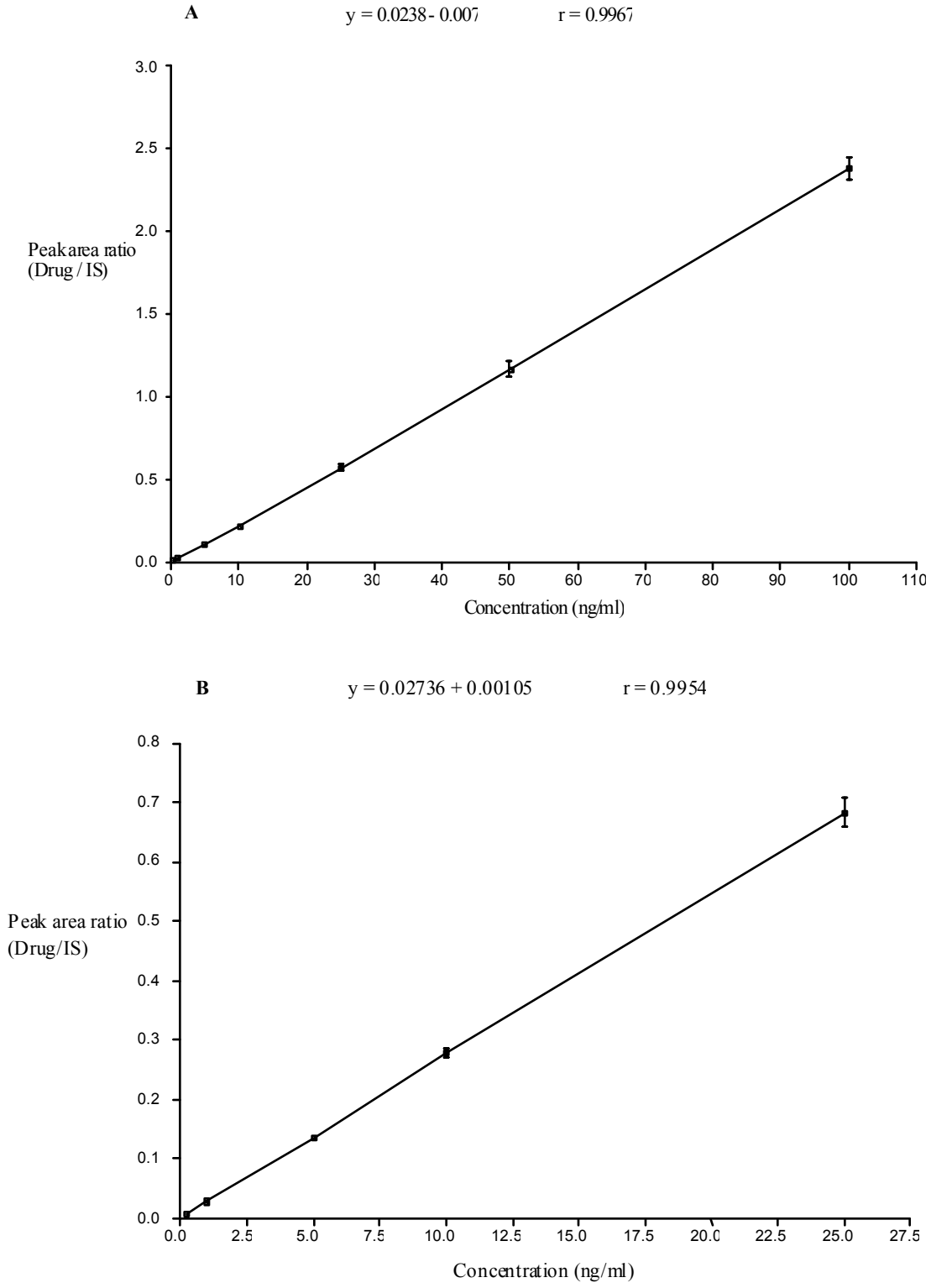


Figure 11. Calibration curves of Tac in human (A) whole blood; and (B) plasma.

A.

Concentration (ng/mL)	100	50	25	10	5	1	0.25
Mean peak area ratio±s.d. (ng/mL)(n=3)	2.34 ± 0.11	1.16 ± 0.05	0.615 ± 0.009	0.224 ± 0.006	0.102 ± 0.009	0.023 ± 0.002	0.004 ± 0.0006

B.

Concentration (ng/mL)	25	10	5	1	0.25
Mean peak area ratio±s.d. (ng/mL)(n=3)	0.687 ± 0.022	0.273 ± 0.015	0.136 ± 0.003	0.025 ± 0.002	0.005 ± 0.0006

Table 14. Mean peak area ratios ± s.d. of the (A) whole blood, and (B) plasma calibration curves shown in Figure 11.

Matrix	**Nominal / theoretical concentration (ng/mL)**	**Assayed concentration (mean ± SD), (ng/mL)**	**CV (%)**	**Accuracy (%)**
Whole blood	0.25	0.29 ± 0.05	17.2	116.0
	1.5	1.64 ± 0.23	14.0	109.3
	15.0	15.15 ± 0.66	4.4	101.0
	75.0	75.47 ± 1.38	1.8	100.6
Plasma	0.25	0.29 ± 0.04	13.8	116.0
	1.5	1.67 ± 0.21	10.8	111.3
	7.5	7.10 ± 0.79	11.1	94.7
	20.0	20.43 ± 1.32	6.46	102.2

Table 15. Intra-day accuracy and precision for quantification of Tac in whole blood and plasma samples at different concentrations.

Matrix	Concentration (ng/mL)	Inter-day variation (CV %)
Whole blood	1.5	15.0
	15.0	9.3
	75.0	2.0
Plasma	1.5	14.2
	7.5	13.3
	20.0	8.3

Table 16. Inter-day precision for Tac quantified in whole blood (n = 18) and plasma (n = 18) samples, respectively.

	Concentration (ng/mL)	Whole blood (mean ± SD)(%)	Concentration (ng/mL)	Plasma (mean ± SD)(%)
Tac	1.5	60.6 ± 8.3	1.5	57.9 ± 4.1
	15.0	59.1 ± 10.0	7.5	69.1 ± 7.2
	75.0	67.1 ± 5.8	20.0	62.8 ± 5.6
Ascomycin	25.0	56.6 ± 5.4	25.0	55.5 ± 7.6

Table 17. Recoveries of Tac and ascomycin from whole blood and plasma (n = 6).

4.3.3. Patients and data collection

Data were collected prospectively from adult and paediatric liver transplant inpatients and outpatients who have been administered Tac at the National University Hospital, Singapore during the period from May 2001 to September 2003. Any such patients were eligible for recruitment into the study. Approval was obtained from the hospital's ethics committee for the study. Informed verbal consent was obtained from the patients or their caregivers for blood sampling in addition to those required for routine TDM.

Adult inpatients were administered oral Tac as part of a triple immunosuppressive regimen that included basiliximab and corticosteroids whereas paediatric inpatients were administered oral Tac as part of a dual immunosuppressive regimen that included corticosteroids. The difference in Tac use (dual versus triple therapy) reflects the different prescribing patterns of Tac during clinical use in different patient groups by the prescribers. Thus the results of this study will be applicable clinically as it was derived from actual clinical data. Therapy was generally initiated at a dose of 0.05 mg/kg and 0.1 mg/kg twice daily for adult and paediatric inpatients, respectively. Subsequent doses were adjusted empirically on the basis of clinical evidence of efficacy and toxicity and to maintain tacrolimus trough blood concentrations between 10 and 15 ng/mL in the first three months post-transplantation and between 5 and 10 ng/mL thereafter (Oellerich *et al.*, 1998).

Serial blood samples were collected from adult and paediatric inpatients within a dosing interval of 12 hours in the immediate post-transplant period. For adult and paediatric outpatients, blood sampling was carried out during one of the outpatient visits for a duration of 2 to 6 hours. Only outpatients who had no changes in the doses of oral Tac for the past 1 week (steady-state) were included in the study. The exact dosing and sampling times were recorded on data collection forms, and this information was used in the analysis. Patient demographic and covariate data were obtained from the hospital's patient medical records. One of the covariates investigated was the erythrocyte-to-plasma (E/P) ratio, i.e. the ratio of Tac concentration in the erythrocytes to that in plasma. The concentration (E) of Tac in the non-plasma, erythrocyte component of whole blood was calculated from the following relationship:

$$\mathrm{E} = \frac{W - P(1 - HCT)}{HCT}$$

where W and P are the concentrations of Tac in whole blood and plasma, respectively (Rowland and Tozer, 1994).

The dataset comprised a total of 213 whole blood Tac concentrations from 16 adult and 15 paediatric liver transplant patients. 182 plasma Tac concentrations were available for the determination of E/P ratio as some of the blood samples were haemolysed and some of the plasma samples were below the limit of quantification of the assay. Table 18 lists the patient characteristics included in this study. There was a wide distribution of the patient covariates from childhood through to adulthood. The common indications for

	Covariate statistics
Continuous covariates	
Demography	
AGE (yr)	40.5 (1.41 – 67.0)
WT (kg)	55.0 (6.5 – 127)
HT (m)	1.61 (0.72 – 1.76)
BSA (m^2)	1.55 (0.363 – 2.45)
Laboratory measurements	
APH (U/L)	149 (37 – 718)
ALT (U/L)	152 (23 – 577)
AST (U/L)	52 (15 – 236)
GGT (U/L)	147 (16 – 1249)
LDH (U/L)	731 (452 – 1453)
TOTBIL (μmol/L)	21 (3 – 309)
ALB (g/L)	32 (24 – 44)
TOTPRO (g/L)	62 (48 – 83)
Alpha-1-acid glycoprotein (AAG) (mg/dL)	87.7 (42.1 – 192.2)
CREA (μmol/L)	60 (15 – 212)
UREA (mmol/L)	6.1 (1.4 – 42.4)
HCT (L/L)	31.1 (22.8 – 46.0)
E/P	68 (12.2 – 147.1)
Dichotomous covariates	
Gender (SEX): M / F	23 / 8
RACE: Chinese / Malay / Indian	23 / 5 / 3
Paediatric / Adult	15 / 16

Table 18. Characteristics of patients (n = 31) included in the study. Values for continuous covariates are given as median (range).

liver transplantation were biliary atresia, hepatitis and alcoholic cirrhosis. Figure 12 depicts the frequency distribution of whole blood samples by sampling time interval.

4.3.4. Data analysis

A graph of the individual Tac concentrations versus time after dose is given in Figure 13. The disposition of Tac appeared to be adequately described by a one-compartment model. Development of the structural PK model indicated that two-compartment model with first order absorption does not significantly improve the description of the observed Tac concentration-time data compared to a linear, open one-compartment model (ΔOBJF = 3.959). For k_a, the inclusion of interindividual variability in the model does not result in a significant improvement.

For residual variance, the lowest OBJF of –1890.693 is produced by the combined additive and proportional error model, followed by the proportional error model (OBJF = -1864.931) and lastly the additive error model (OBJF = -1863.775). However, the CV associated with the estimation of the proportional portion of the combined additive and proportional error model is very high (i.e. 91.3 %). Thus, this residual error model is not selected for use and the proportional error model is selected instead.

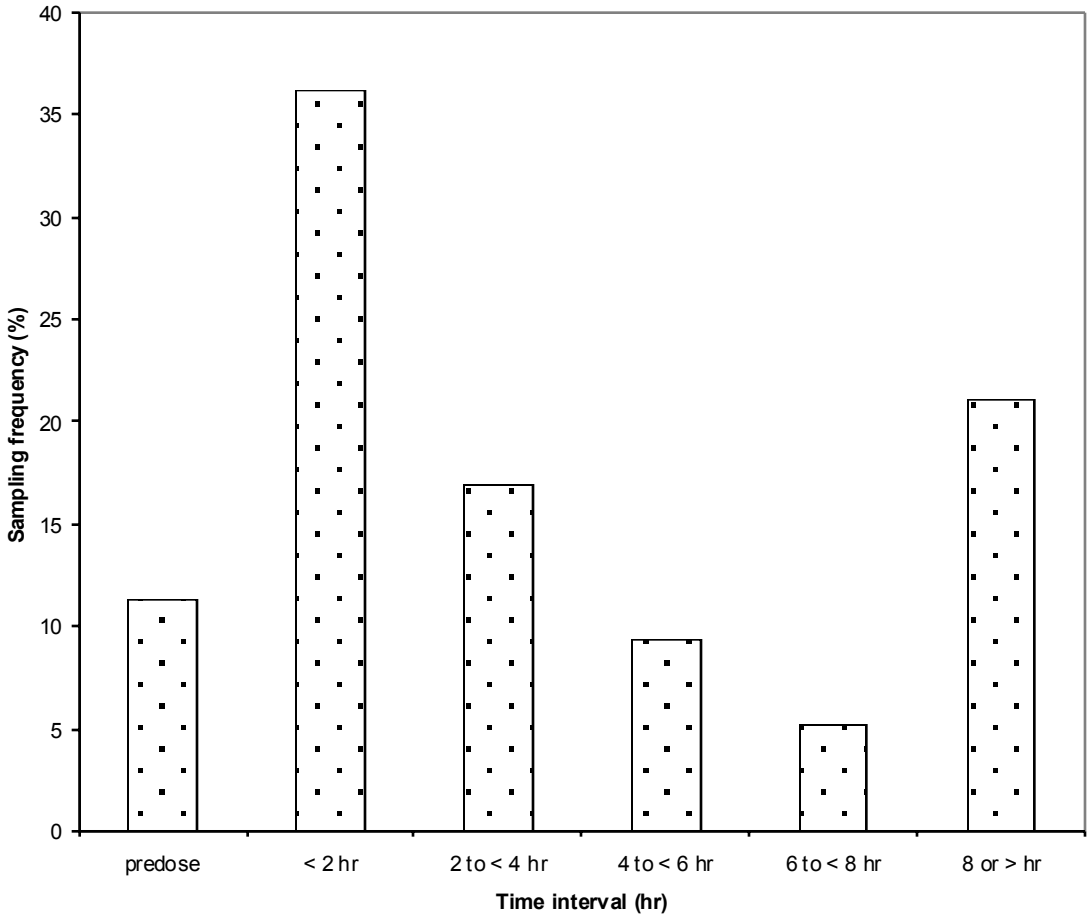


Figure 12. Frequency distribution of whole blood samples by collection time interval.

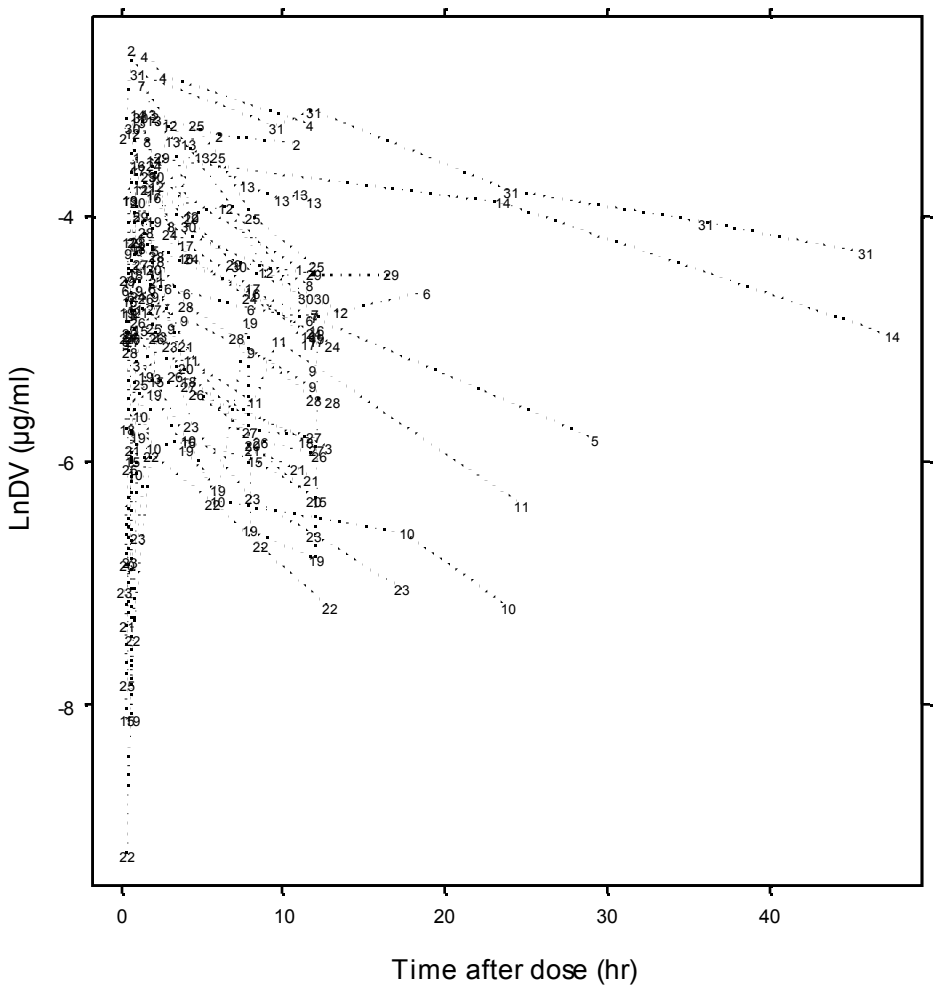


Figure 13. Observed whole blood Tac concentrations (DV) vs time after dose (TAD) plotted on a semilogarithmic scale. Data points are labeled by the ID number and each individuals data points are connected.

A one-compartment PK model with first order absorption (subroutines ADVAN 2 TRANS 2) is thus chosen as the basic structural model. At step 1, large interindividual variability was observed in CL/F (104.4 %) and V/F (113.1 %), suggesting that accounting for individual characteristics could reduce the variability and refine the predictive model. Basic goodness of fit plots for the basic structural sub-model of the population model are shown in Figure 14.

In step 2 of model development, the GAM analysis indicated that Tac CL/F is a linear function of WT, and V/F is a linear function of HT, CREA and HCT. Tables 19 and 20 summarize the path taken to these models and show some of the other GAMs that are close to the best fit one. In each step, the term that results in the largest decrease in the AIC is added to the model. The residual sum of squares (RSS) and changes in RSS from the previous model are also shown. WT has the greatest influence on CL/F and it alone explains 35.6 % of the observed variability in CL/F. HT has the greatest influence on V/F, followed by CREA and HCT, accounting for 20.5 %, 19.4 %, and 14.9 % of the observed variability in V/F, respectively. The relationship between WT and Tac CL/F for the final GAM is shown in Figure 15. Figure 16 shows an Akaike plot for the GAM run for CL/F. The relationships between covariates and Tac V/F for the final GAM are shown in Figure 17. Figure 18 shows an Akaike plot for the GAM run for V/F.

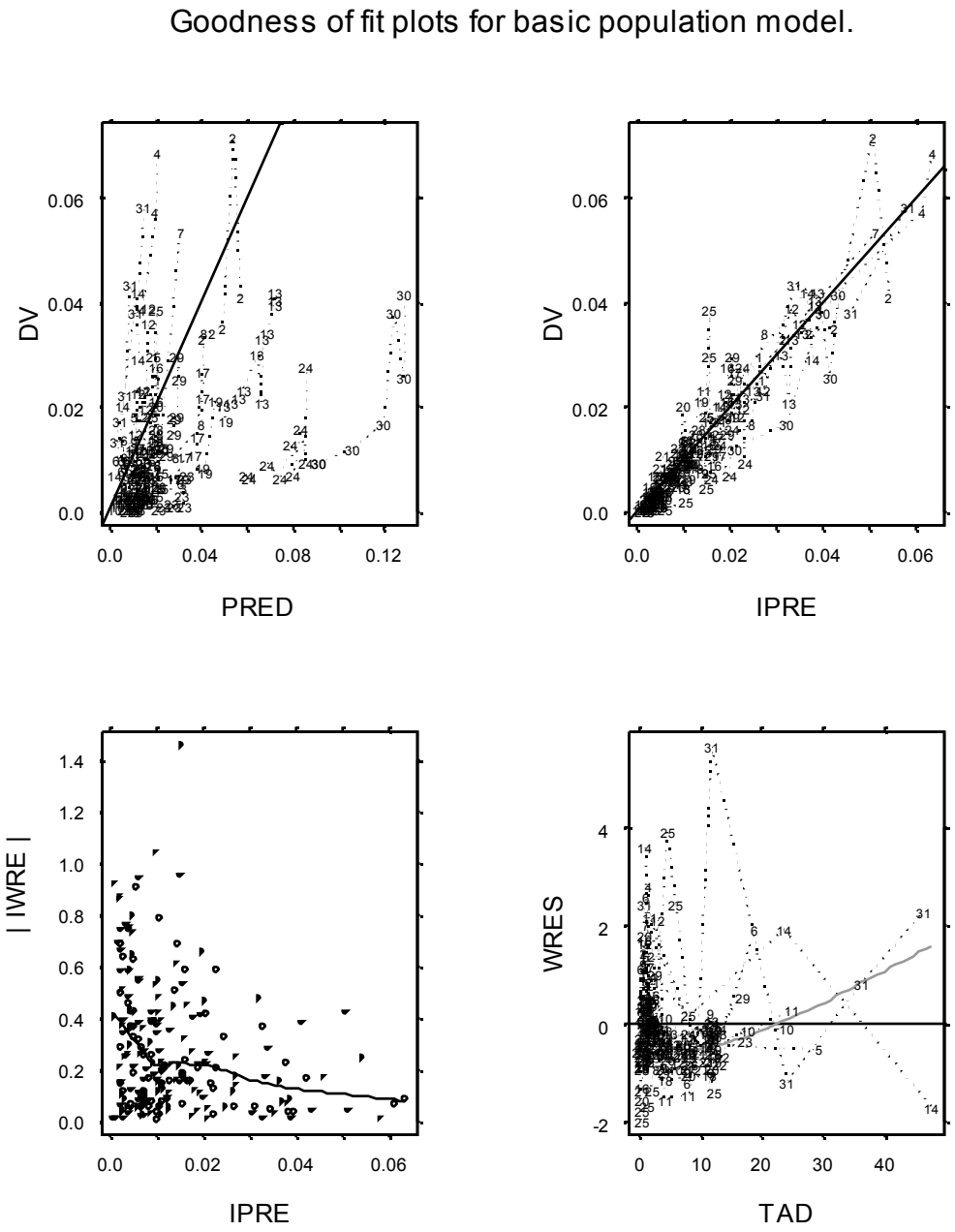


Figure 14. Basic goodness of fit plots for the basic population model. The upper left panel shows the predictions for the typical individual in the population (PRED) vs the dependent variable (DV), i.e. the observed concentrations. The upper right panel is a plot of the individual predictions (IPRE) vs the dependent variable. The lower left is a plot of the absolute values of the individual weighted residuals (IWRE) vs the individual predictions. The lower right panel is a plot of the weighted residuals (WRES) vs the independent variable (TIME AFTER DOSE, TAD). In all but the lower left panel, each individual data points are connected by a line and labeled with the ID number. In the uppermost two panels, the solid line is the line of identity. In the lower left panel, the solid line is a smooth.

Step	Term	ΔRSS	RSS	AIC
1	constant		1145.5	1221.9
2	+ WT	-407.7	737.8	890.6

Summary of models close to the minimum AIC model

Equation	AIC
CL/F ~ WT	891
CL/F ~ BSA	895
CL/F ~ WT + HCT	918

Table 19. The top part of the table shows the path taken to the final GAM for the CL/F of Tac, starting with the NULL model. In each step, the term that results in the largest decrease in the AIC is added (+) to the model. The bottom part of this table shows some models close to the final GAM (model with the smallest AIC value). The ~ in the notation of the models means "is a function of" and the + signs are to be interpreted figuratively and not literally.

Step	Term	ΔRSS	RSS	AIC
1	constant		1773233	1891448
2	+ HT	-362684	1410549	1646980
3	+ CREA	-344668	1065881	1420527
4	+ HCT	-263901	801980	1274842

Summary of models close to the minimum AIC model

Equation	AIC
V/F ~ HT + CREA + HCT	1270000
V/F ~ HT + CREA + UREA + HCT	1360000
V/F ~ ns(HT, df=2) + CREA + HCT	1362000

Table 20. The top part of the table shows the path taken to the final GAM for the V/F of Tac, starting with the NULL model. In each step, the term that results in the largest decrease in the AIC is added (+) to the model. The bottom part of this table shows some models close to the final GAM (model with the smallest AIC value). The ~ in the notation of the models means "is a function of" and the + signs are to be interpreted figuratively and not literally.

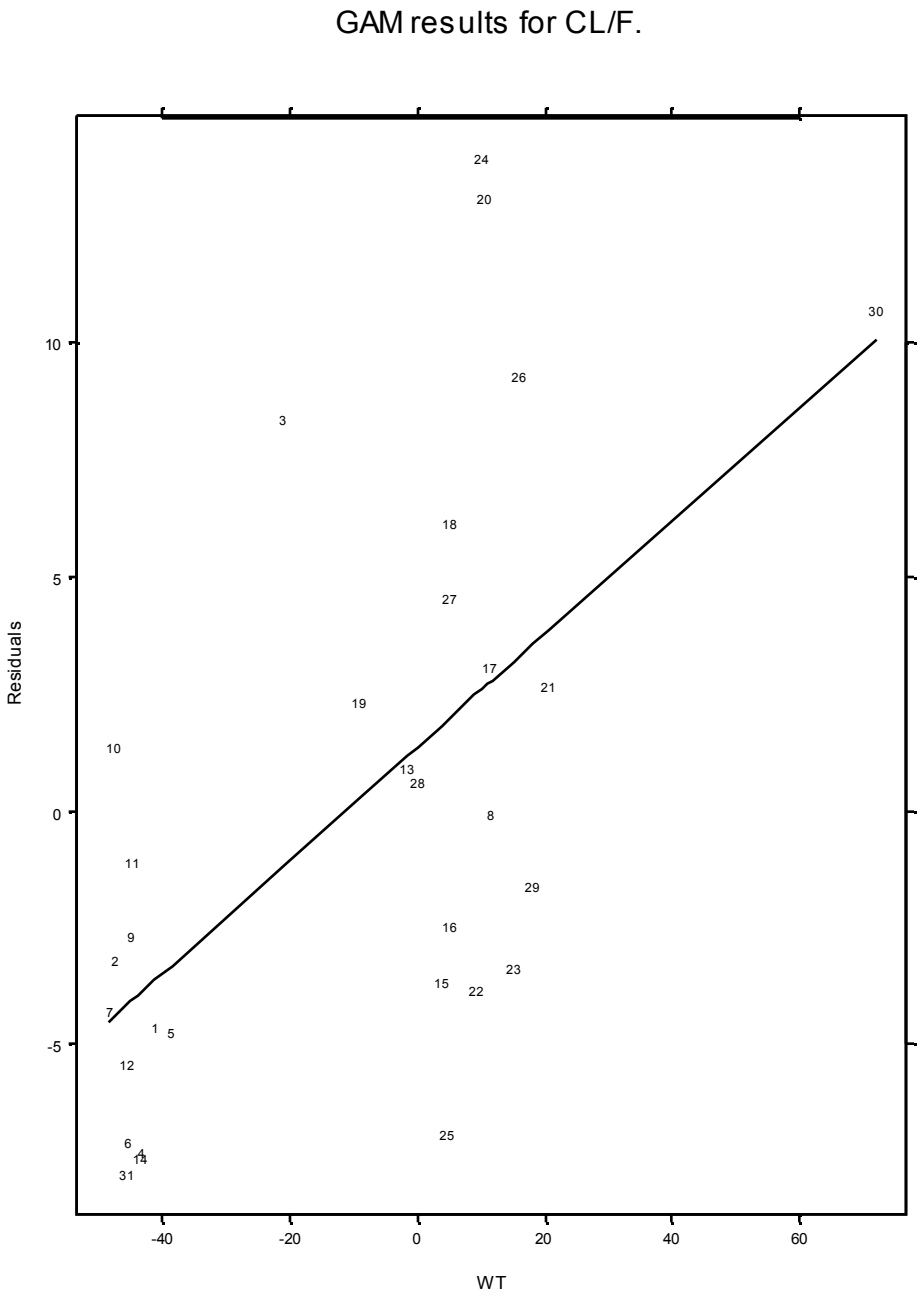


Figure 15. Results of GAM for CL/F. On the y-axis are the residuals from the GAM fit and on the x-axis is WT-55. Each individual point is labeled with an ID number.

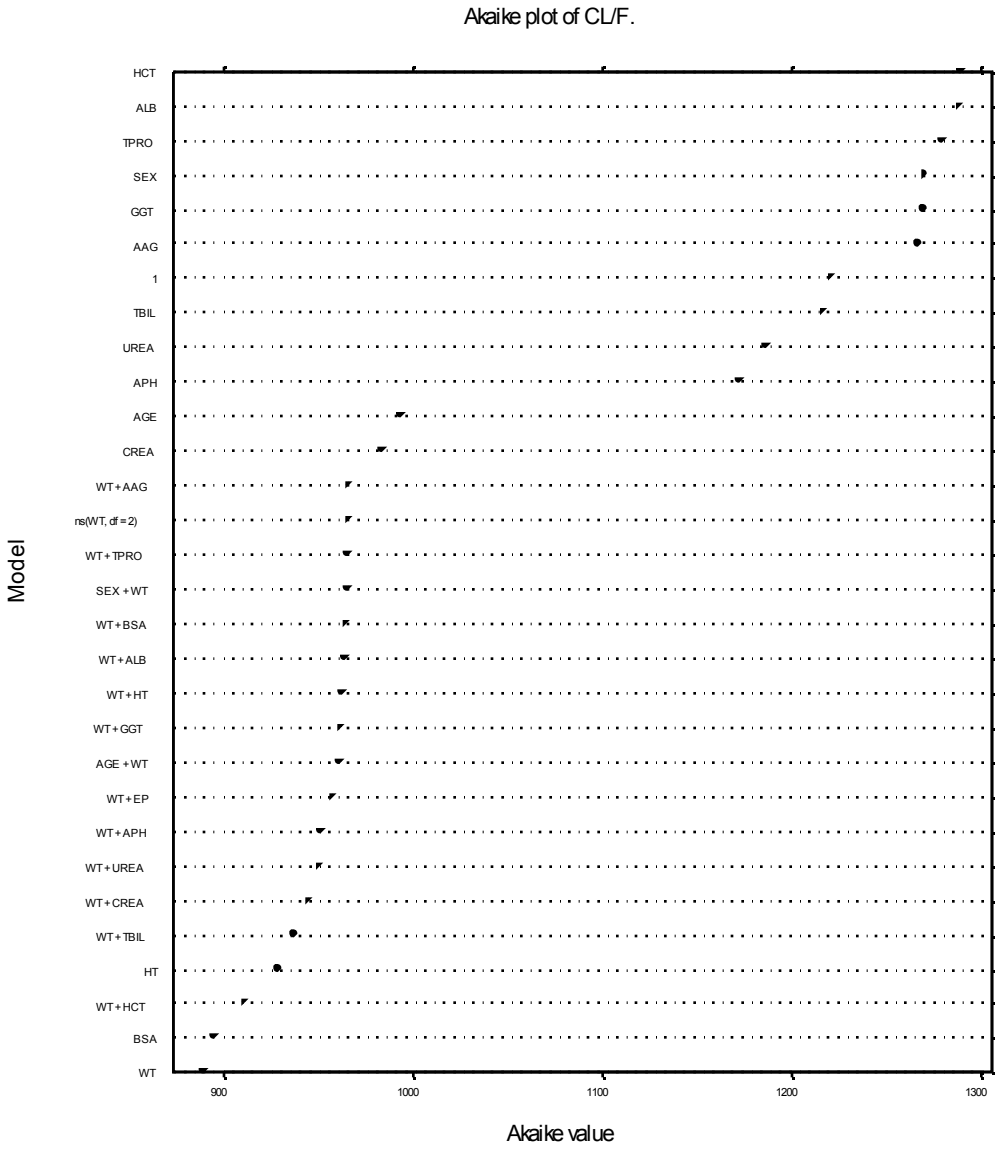


Figure 16. Results of GAM for CL/F, showing the Akaike plot of CL/F. On the y-axis are the models tested, with the best model at the bottom. The plus sign in some of the models indicates that the model tested consisted of the two terms, i.e. not addition. On the x-axis is the Akaike value, the lower the Akaike value, the better the fit.

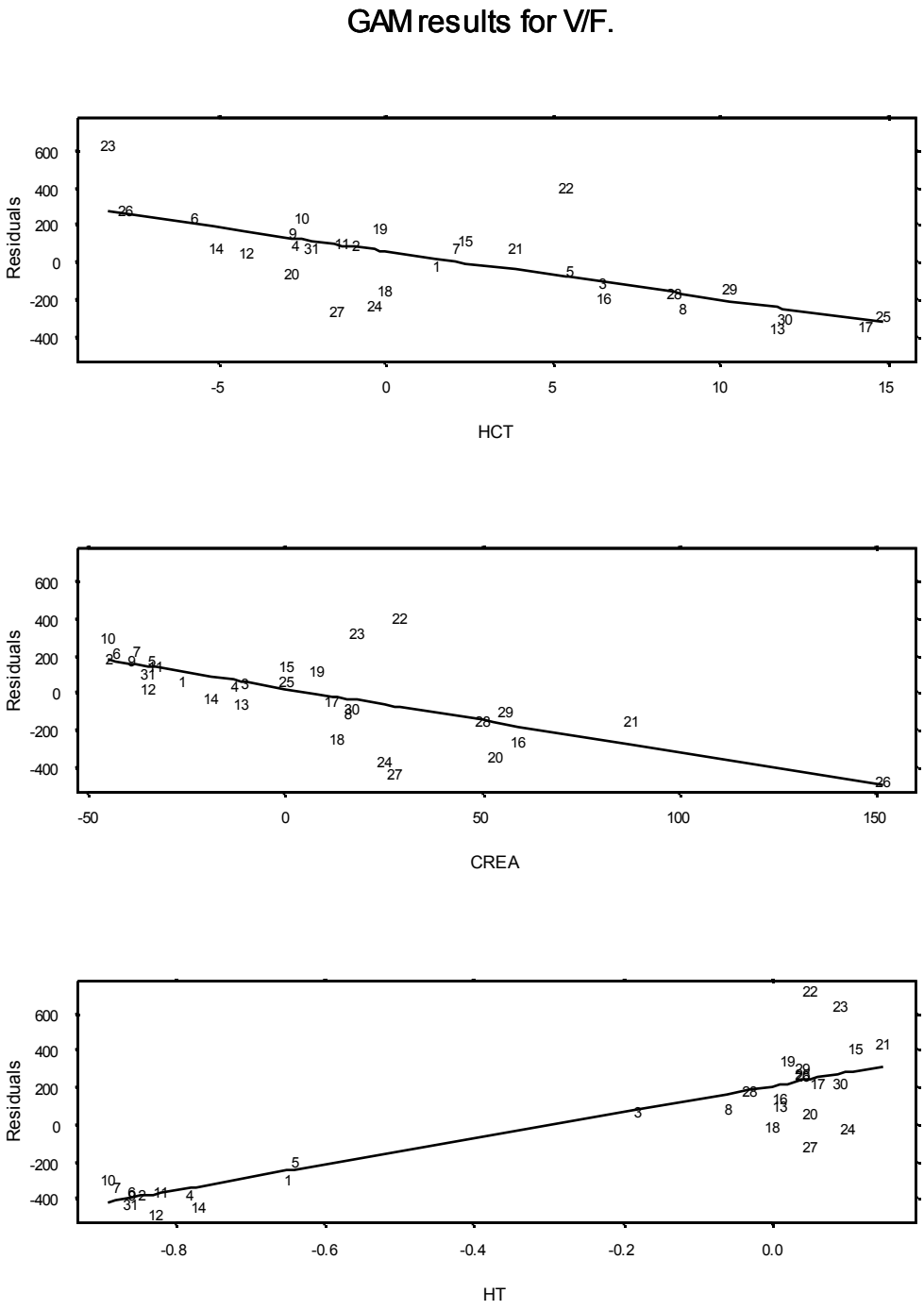


Figure 17. Results of GAM for V/F. On the y-axes on each of the plots are the residuals from the GAM fit. On the x-axis of the uppermost plot is HCT-31.1. On the x-axis of the middle plot is CREA-60. On the x-axis of the lowermost plot is HT-1.61.

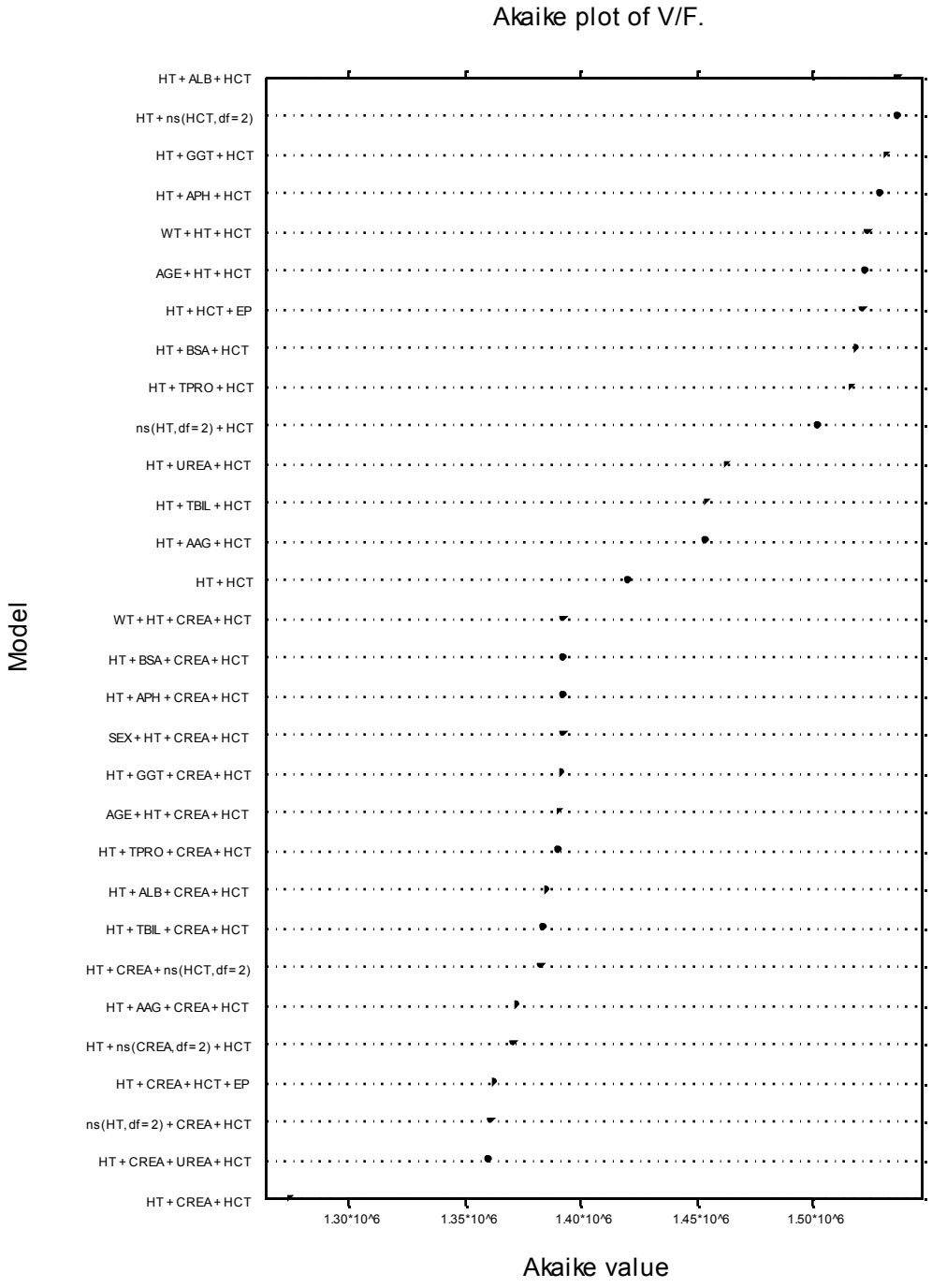


Figure 18. Results of GAM for V/F, showing the Akaike plot of V/F. On the y-axis are the models tested, with the best model at the bottom. The plus sign in some of the models indicates that the model tested consisted of the two terms, i.e. not addition. On the x-axis is the Akaike value, the lower the Akaike value, the better the fit.

Bootstrap of the GAM for CL/F shows that WT has the highest relative inclusion frequency of 0.44 amongst the covariates investigated (Figure 19). The relative inclusion frequency was obtained by counting the number of times each covariate was selected by the GAM divided by the number of iterations. Therefore, the results derived from the bootstrap data supported the results from the original GAM analysis conducted with the actual patient data. It also shows that WT is indeed the most stable covariate relationship (Figure 20). Approximately 56 % of the 150 bootstrapped data sets had a covariate model size equal to two (Figure 21) in the final GAM model. To assess the adequacy of the number of bootstrapped data set used, a plot of the relative inclusion frequencies vs the bootstrap iterations is made (Figure 22). This plot shows that the frequency of inclusion of the covariates stabilizes after about 100 bootstrap iterations. Thus the use of 150 bootstrap datasets in bootstrap of the GAM is suitable.

Bootstrap of the GAM for V/F shows that HCT, CREA and HT have the highest relative inclusion frequencies of 0.59, 0.53 and 0.48, respectively, amongst the covariates investigated (Figure 23). Therefore, the results derived from the bootstrap data supported the results from the original GAM analysis conducted with the actual patient data. It also shows that these three covariates are the most common three covariate combination (Figure 24). Approximately 66 % of the 150 bootstrapped data sets had a covariate model size equal to three (Figure 25) in the final GAM model. To assess the adequacy of the number of bootstrapped data set used, a plot of the relative inclusion frequencies vs the bootstrap iterations is made (Figure 26). This plot shows that the frequency of inclusion

of the covariates stabilizes after about 100 bootstrap iterations. Thus the use of 150 bootstrap datasets in bootstrap of the GAM is suitable.

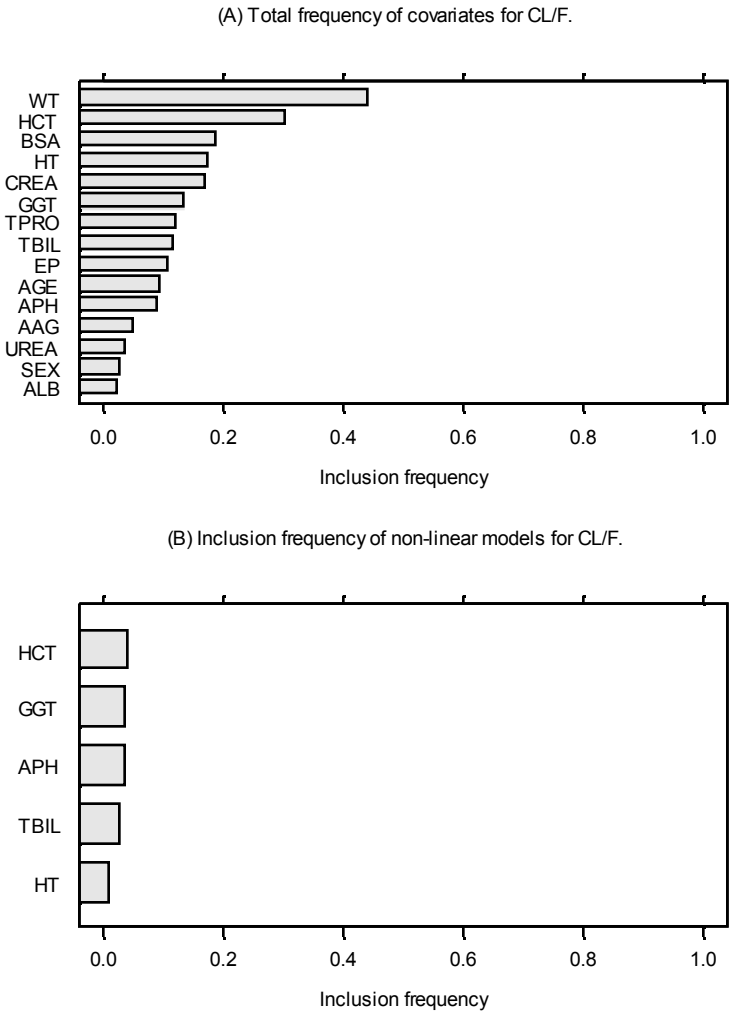


Figure 19. Results of bootstrap of the GAM for CL/F, showing (A) Total frequency of covariates for CL/F, and (B) Inclusion frequency of non-linear models for CL/F.

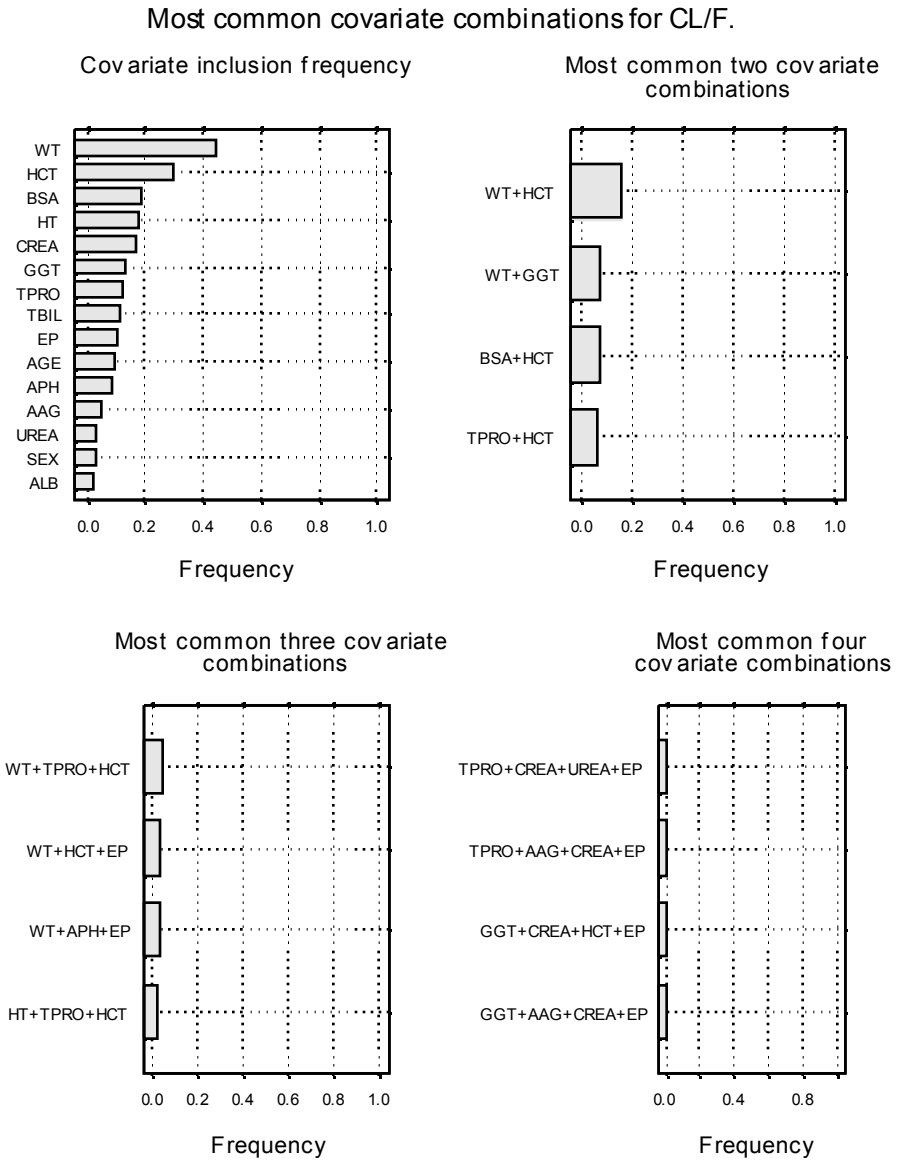


Figure 20. Results of bootstrap of the GAM for CL/F, showing the most common one to four covariate combinations for CL/F. The y-axes shows the covariate combinations and the x-axes the relative inclusion frequency, i.e. the number of times the combination was found divided by the number of iterations (150).

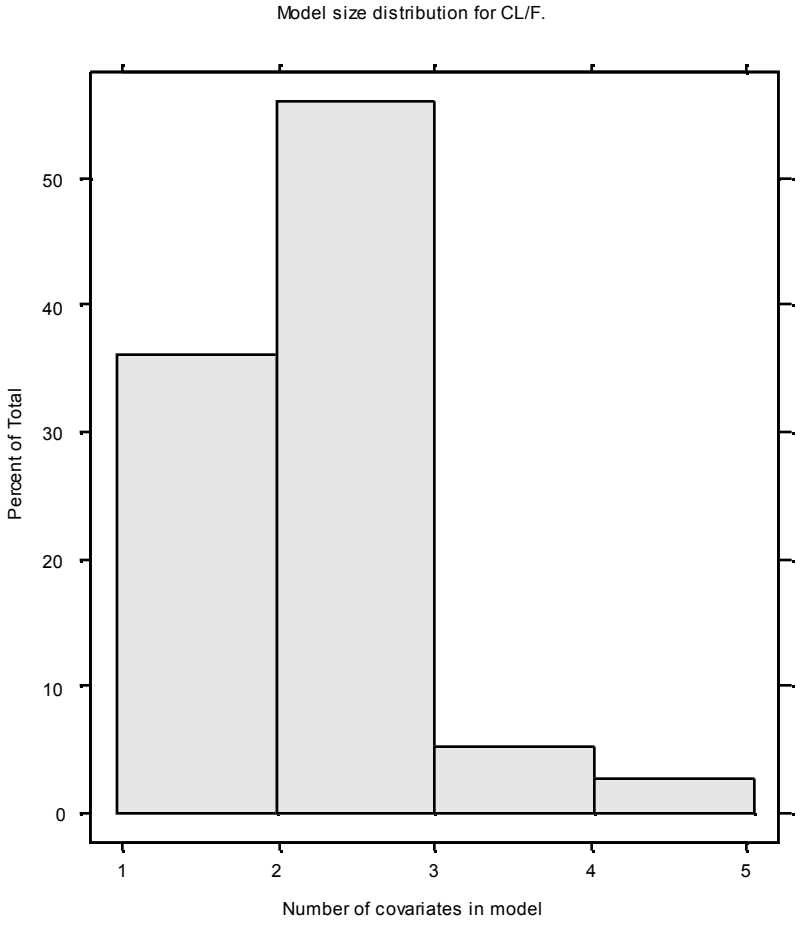


Figure 21. Results of bootstrap of the GAM for CL/F, showing model size distribution for CL/F.

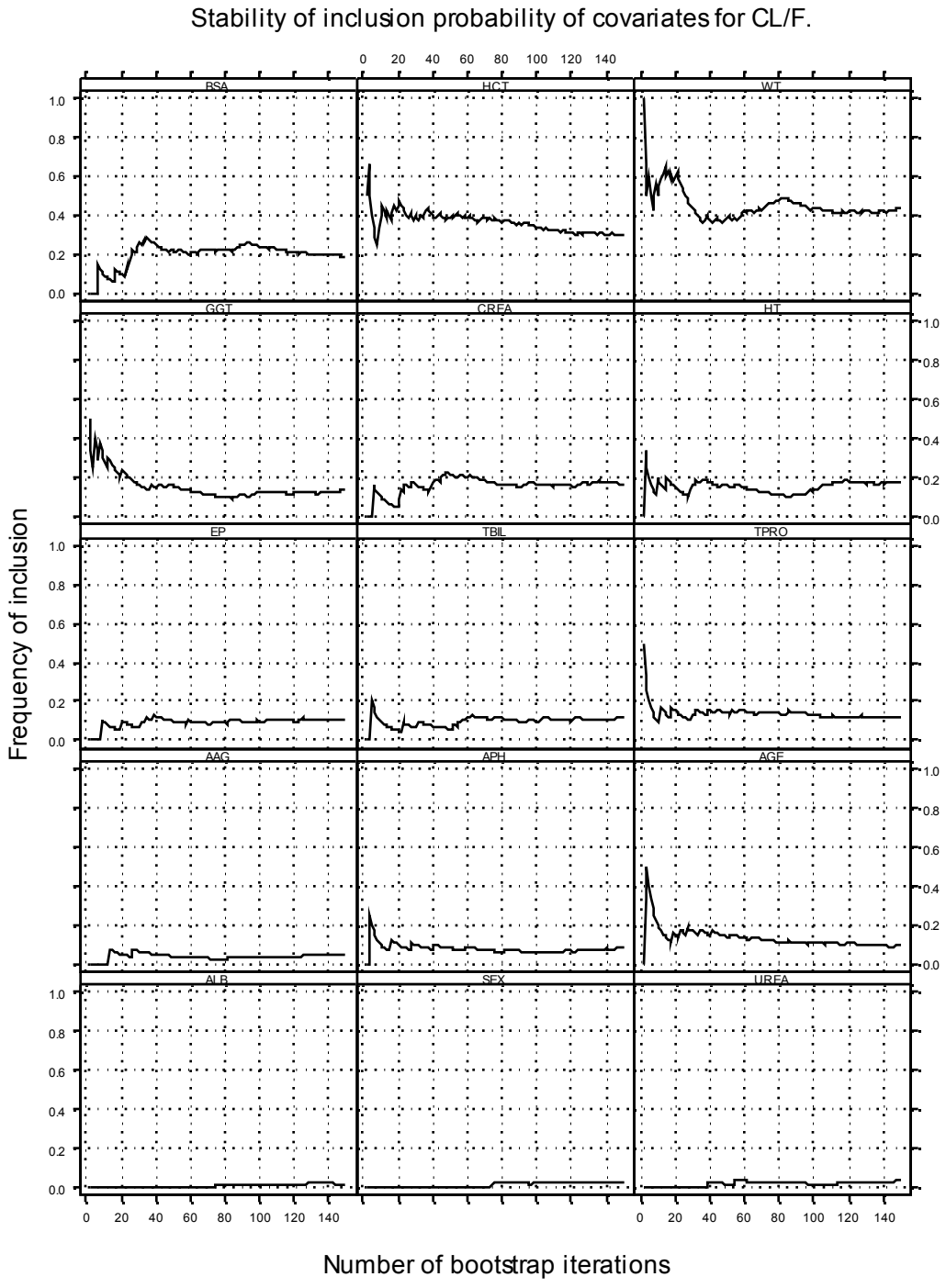


Figure 22. Results of bootstrap of the GAM for CL/F, showing stability of inclusion probability of covariates for CL/F.

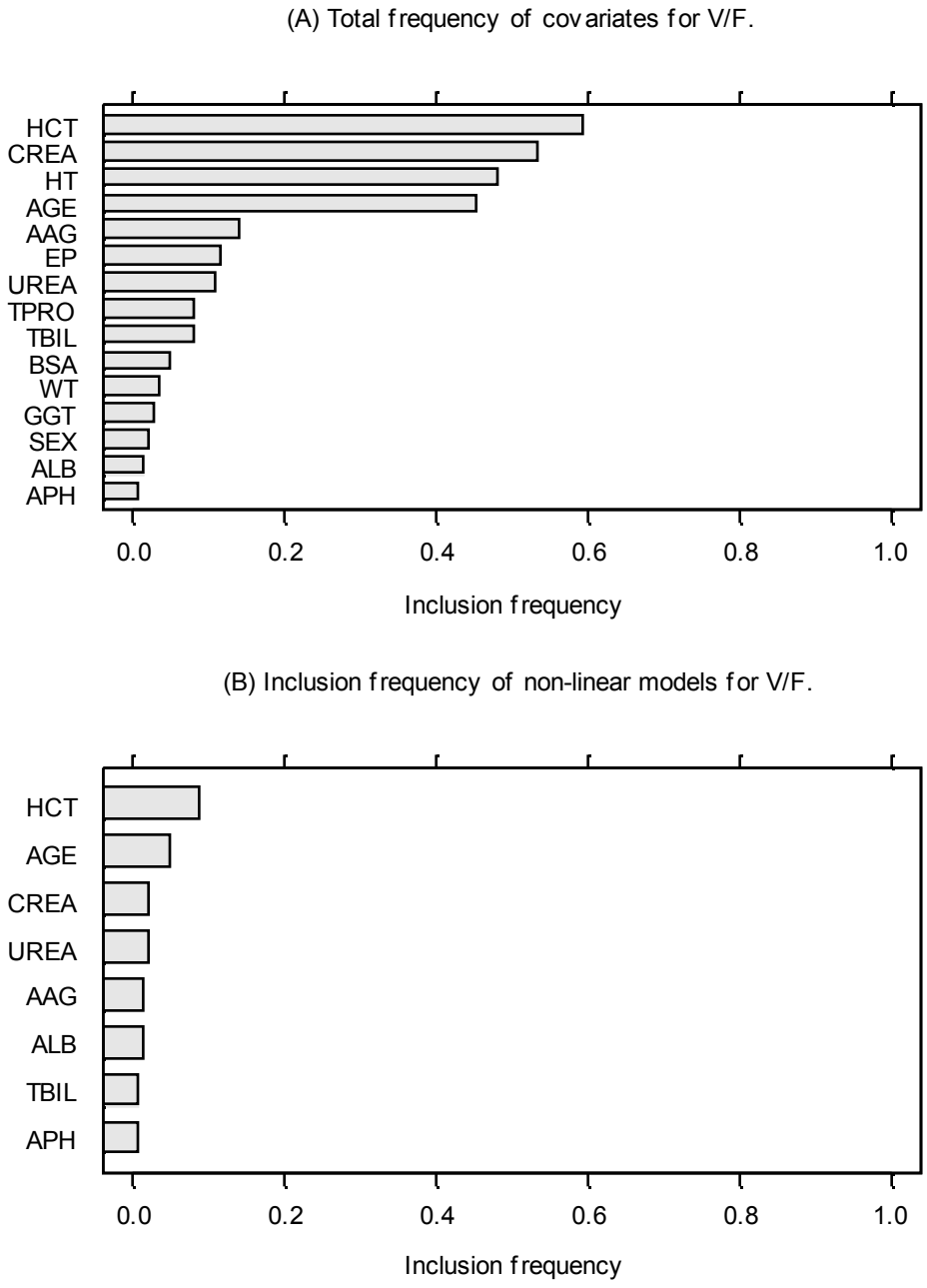


Figure 23. Results of bootstrap of the GAM for V/F, showing (A) Total frequency of covariates for V/F, and (B) Inclusion frequency of non-linear models for V/F.

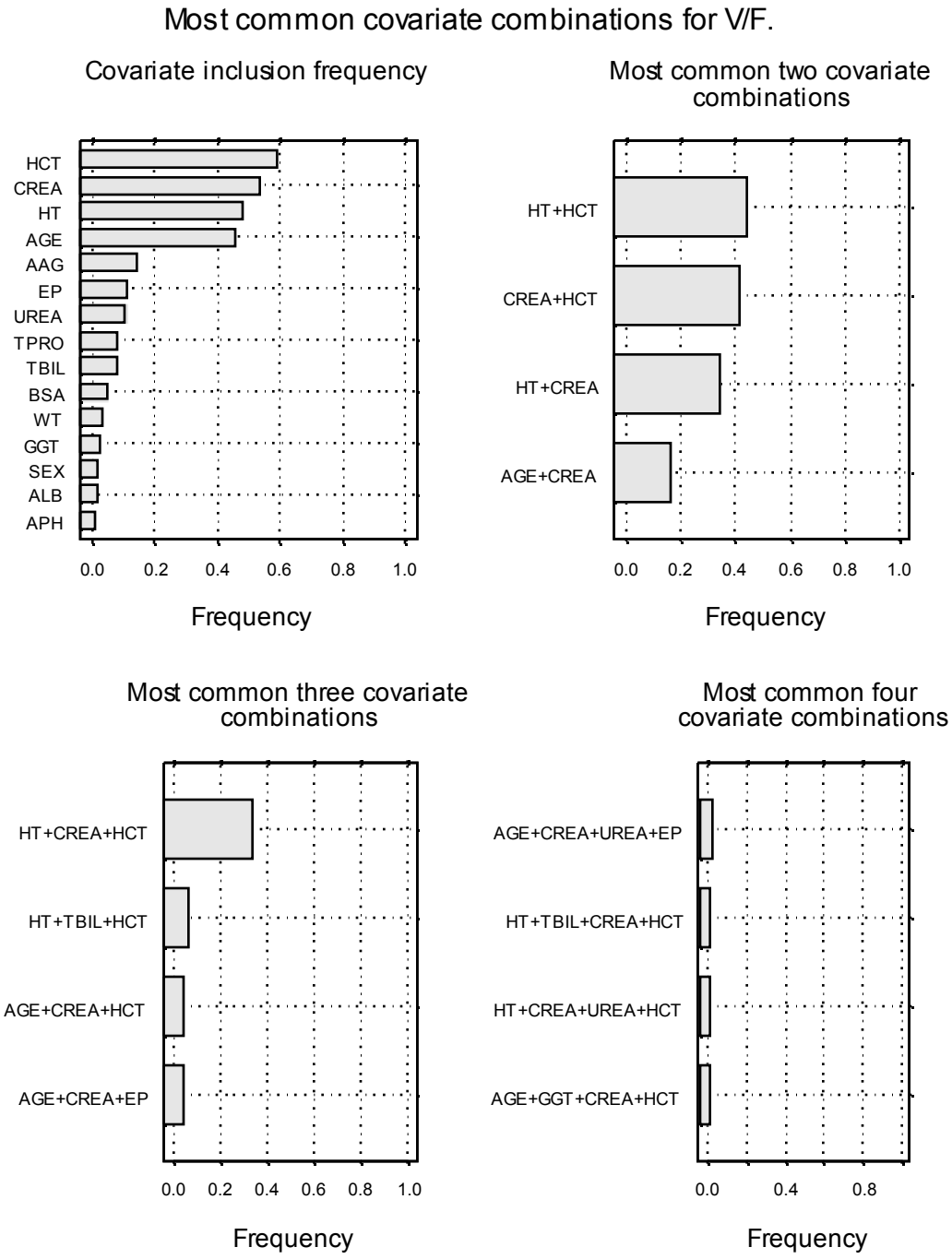


Figure 24. Results of bootstrap of the GAM for V/F, showing the most common covariate combinations for V/F. The y-axes shows the covariate combinations and the x-axes the relative inclusion frequency, i.e. the number of times the combination was found divided by the number of iterations (150).

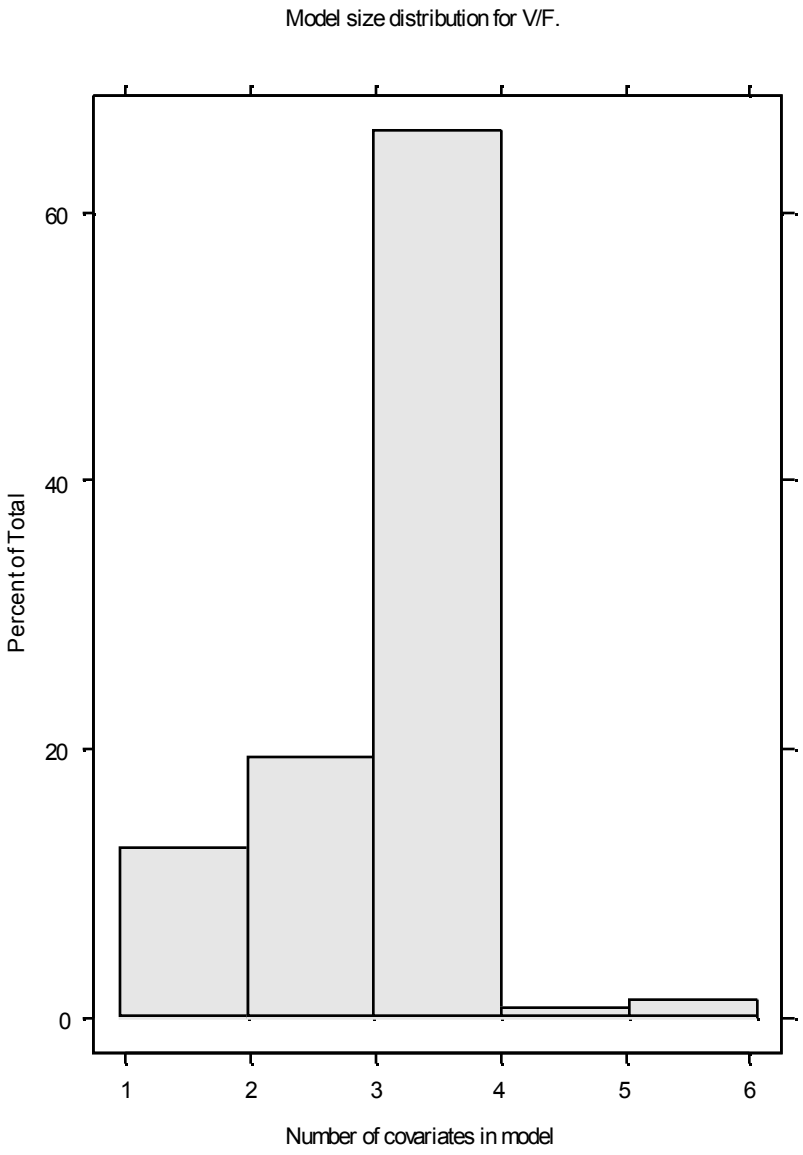


Figure 25. Results of bootstrap of the GAM for V/F, showing the model size distribution for V/F.

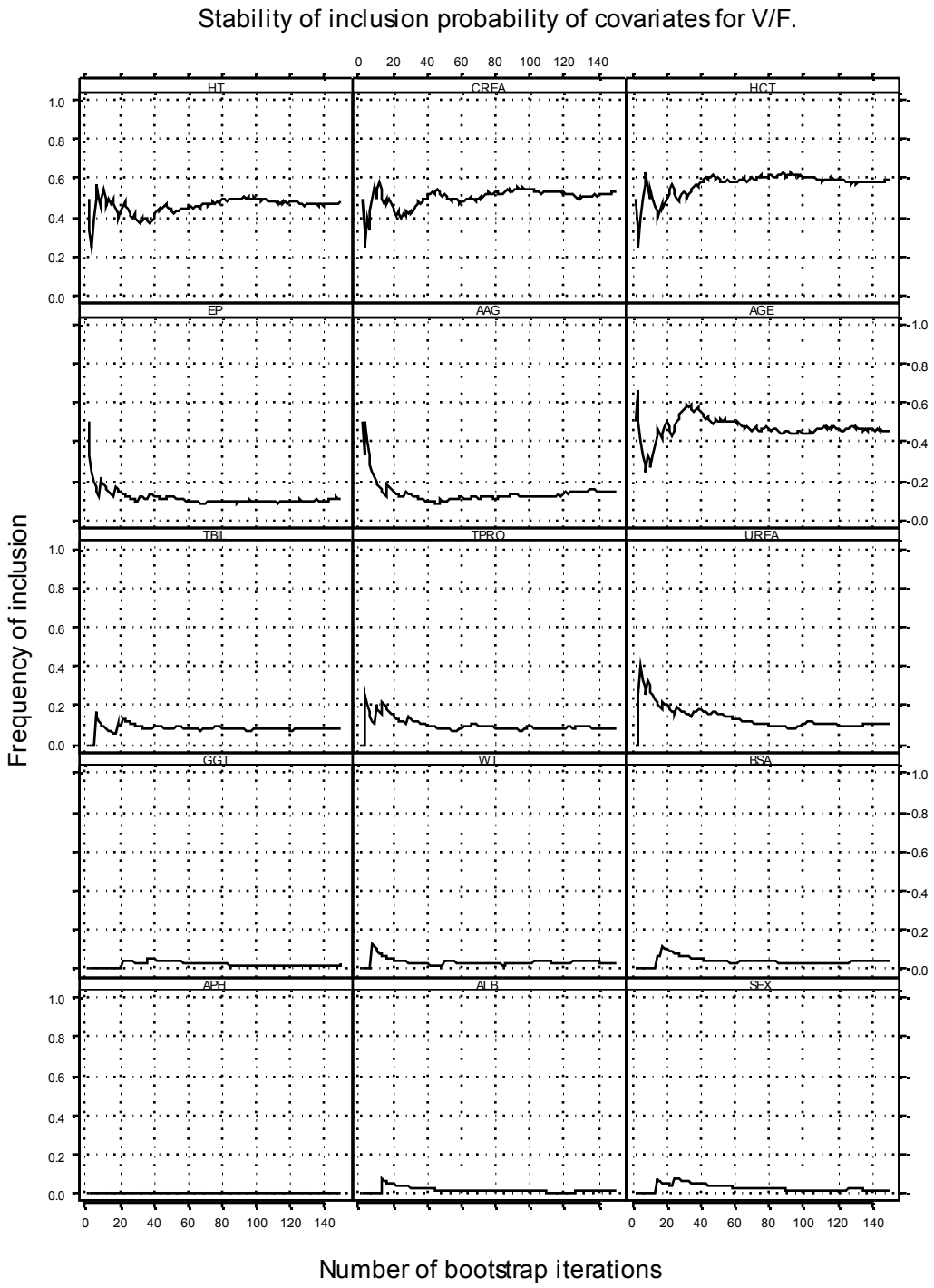


Figure 26. Results of bootstrap of the GAM for V/F, showing the stability of inclusion probability of covariates for V/F.

For TBM, the "unpruned" regression tree (size 5) for CL/F is shown in Figure 27. Cross validation studies show an optimal tree size of 3 for this regression tree (Figure 28). The "pruned" tree of size 3 for CL/F is shown in Figure 29, which shows that WT and HCT are the predictors of CL/F. The first and second nodes for splitting the data occurred at WT of 25.2 kg and HCT of 32.3 L/L, respectively.

For TBM, the "unpruned" regression tree (size 4) for V/F is shown in Figure 30. Cross validation studies show an optimal tree size of 2 for this regression tree (Figure 31). The "pruned" tree of size 2 for V/F is shown in Figure 32, which shows that HT is the most significant predictor of V/F. The node for splitting the data occurred at HT of 1.625 m.

The results of the hypothesis testing on the covariates using NONMEM are summarized in Table 21. APH was included in the covariate model for CL/F as a discrete covariate with 200 U/L as the cut-off value instead of a continuous variable, because it produced better parameter estimates in the model. Table 22 summarizes the ΔOBJF when each of the Θs of the covariates appearing in the final NONMEM model is set to zero, showing the significance of each of the parameters (a $\Delta OBJF > 10.8$ is significant with $p < 0.001$). A comparison of the covariates selected by the different regression methods and NONMEM is given in Table 23. From this table, it can be seen that there was no uniform agreement in the covariate sets selected by the regression methods and NONMEM. However, WT was consistently selected for the covariate model for CL/F while HT was consistently selected for the covariate model for V/F by the three methods.

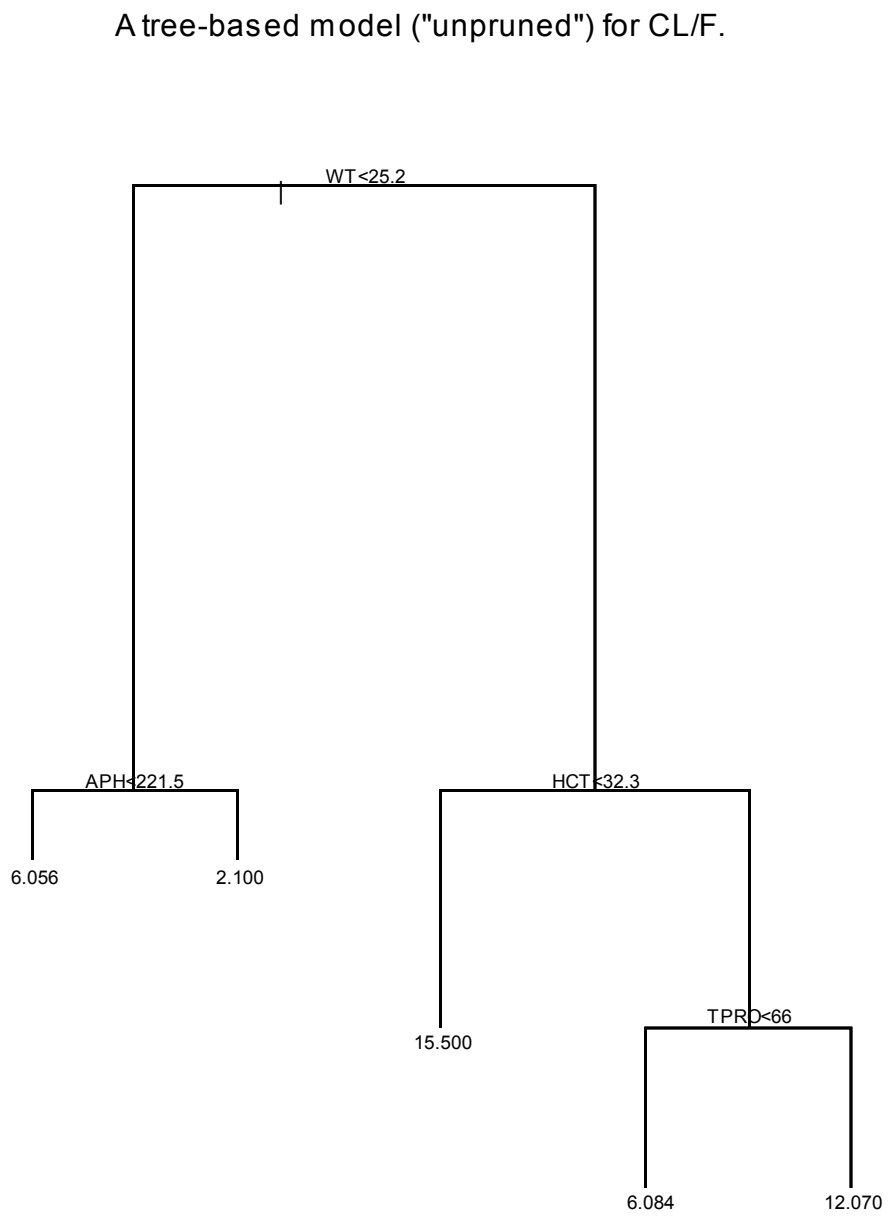


Figure 27. Regression tree ("unpruned") of CL/F. The partitioning variable and its value are presented above to each split. The length of each branch is proportional to the importance of the split. At the terminal leaves, the predicted values of the dependent variable are displayed.

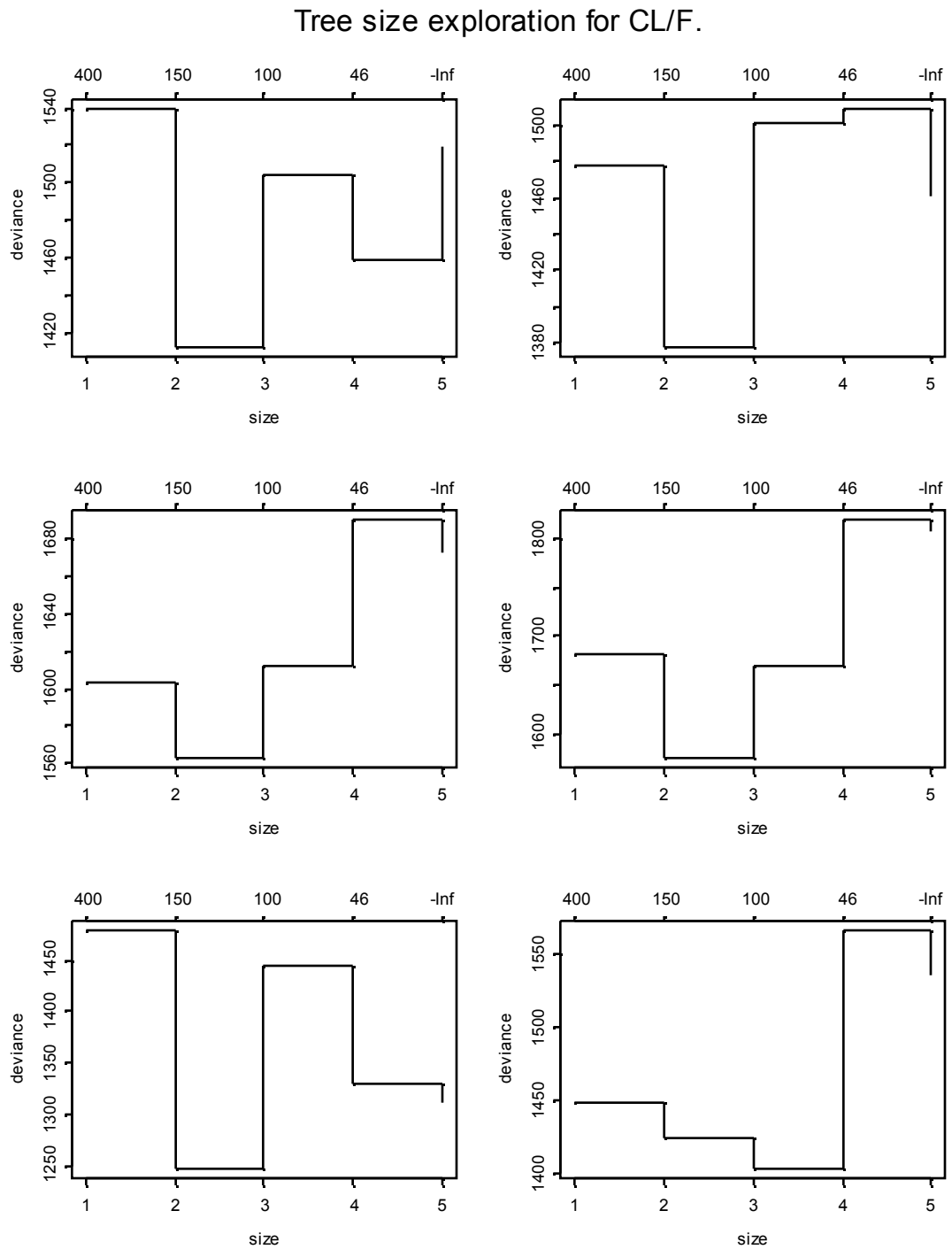


Figure 28. Results from exploring the optimal tree size using cross-validation. On the y-axes are the deviances and on the x-axes are the corresponding tree sizes.

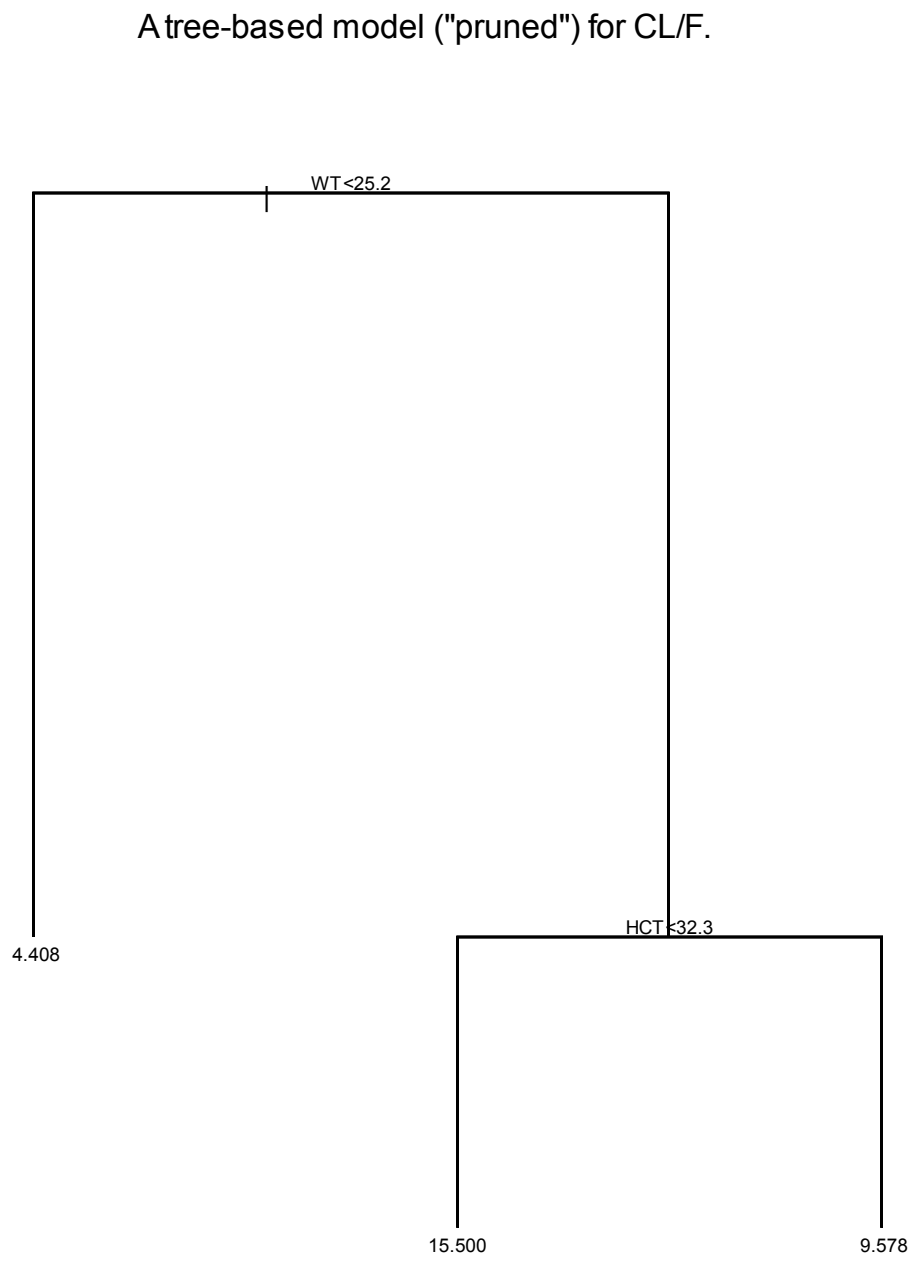


Figure 29. Regression tree ("pruned", size 3) of CL/F. The partitioning variable and its value are presented above to each split. The length of each branch is proportional to the importance of the split. At the terminal leaves, the predicted values of the dependent variable are displayed.

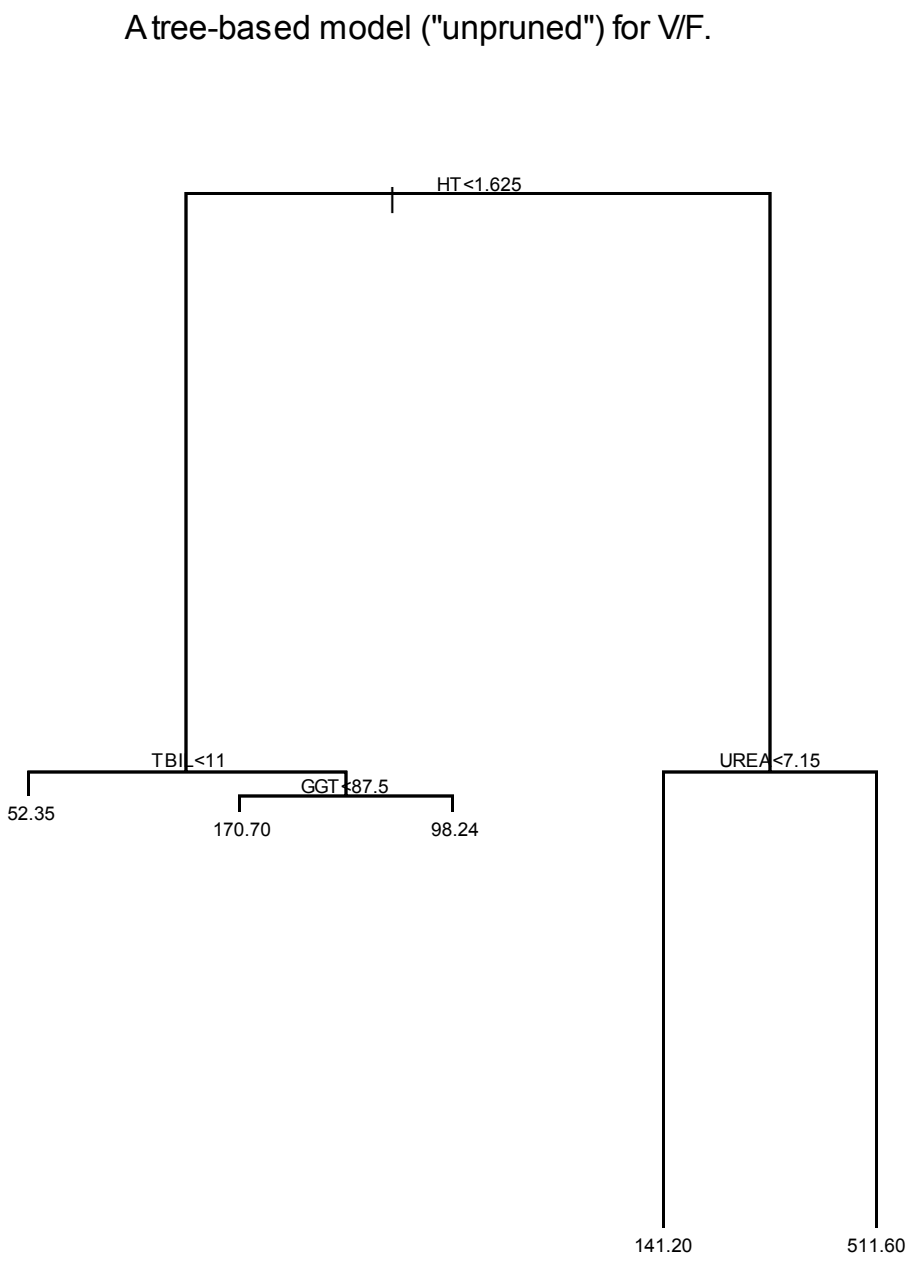


Figure 30. Regression tree ("unpruned") of V/F. The partitioning variable and its value are presented above to each split. The length of each branch is proportional to the importance of the split. At the terminal leaves, the predicted values of the dependent variable are displayed.

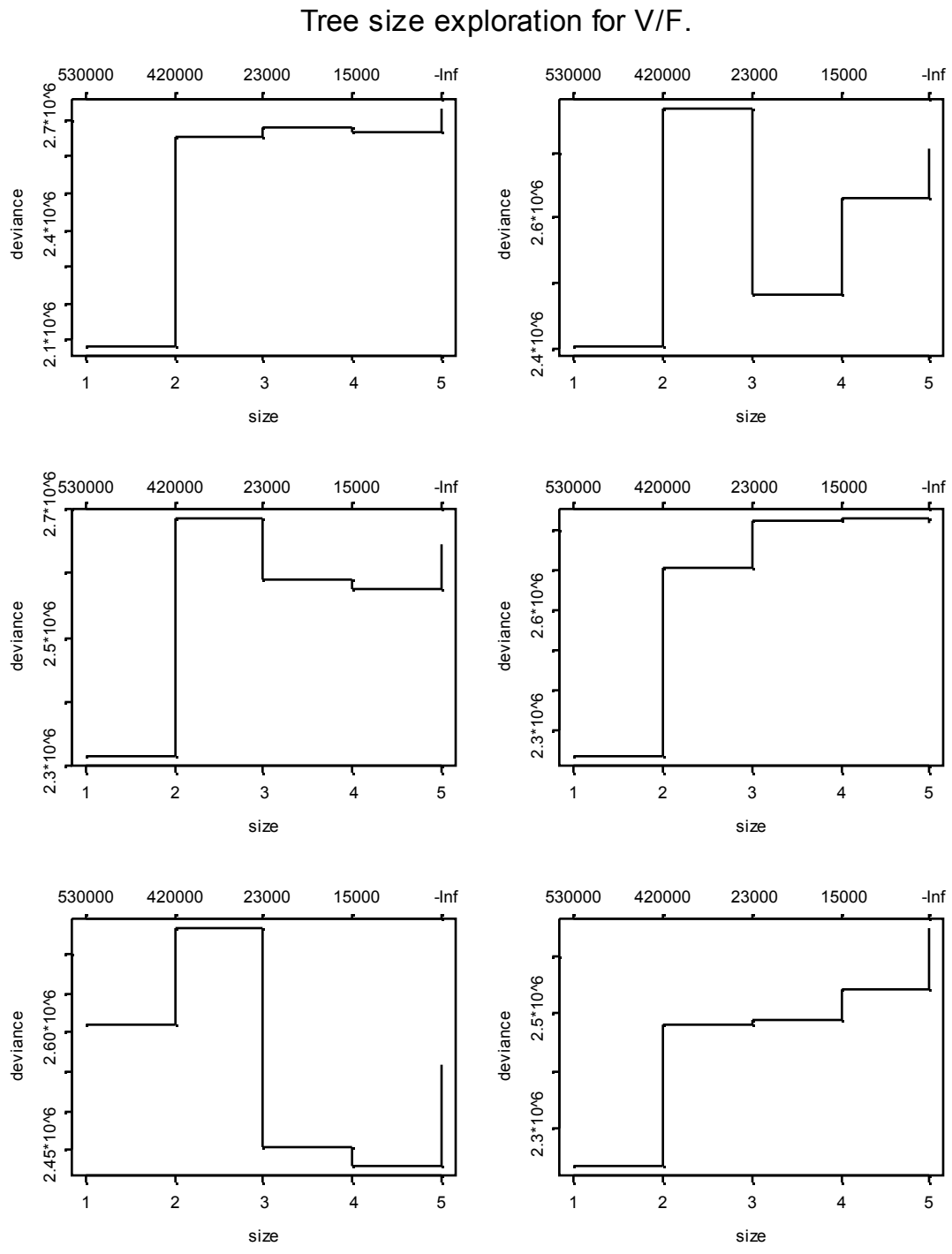


Figure 31. Results from exploring the optimal tree size using cross-validation. On the y-axes are the deviances and on the x-axes are the corresponding tree sizes.

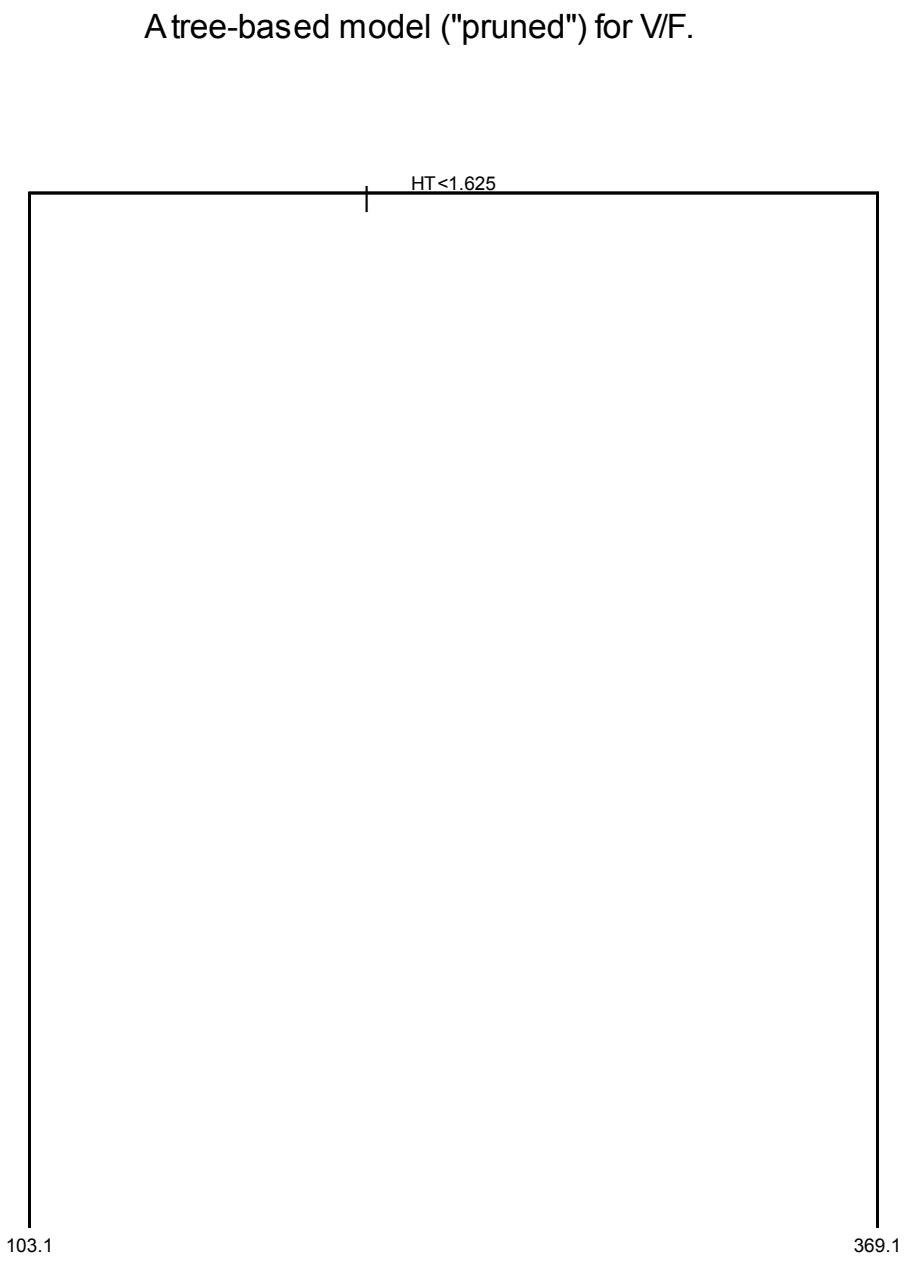


Figure 32. Regression tree ("pruned", size 2) of V/F. The partitioning variable and its value are presented above to each split. The length of each branch is proportional to the importance of the split. At the terminal leaves, the predicted values of the dependent variable are displayed.

Table 21. Summary of the results on the principal PK models tested (step-up procedure).

Model no.[a]	Hypothesis tests	OBJF	ΔOBJF	Ref.	p-value
[1]	CL/F, V/F, k_a	-1864.931			
[2]	CL/F=F(WT)	-1966.314	101.383	vs. [1]	<0.001
[3]	CL/F=F(WT), V/F=(HCT)	-1973.465	7.151	vs. [2]	<0.01
[4]	CL/F=F(WT), V/F=(HCT, HT)	-2009.909	36.444	vs. [3]	<0.001
[5]	CL/F=F(WT, APH), V/F=(HCT, HT)	-2026.264	16.355	vs. [4]	<0.001
[6]	CL/F=F(WT, APH, CREA), V/F=(HCT, HT)	-2038.14	11.876	vs. [5]	<0.001[b]

[a]Exponential intersubject variability for structural PK parameters of CL/F and V/F.
[b]Final model.

CL/F model		V/F model	
Covariate	**ΔOBJF**	**Covariate**	**ΔOBJF**
WT	41.991	HT	36.705
APH	12.406	HCT	26.024
CREA	11.876		

Table 22. ΔOBJF when each of the Θs of the covariates appearing in the final NONMEM model for Tac is set to zero (step-down procedure).

	Regression methods		
Parameter	**GAM**	**TBM**	**NONMEM**
CL/F	~ WT	~ WT + HCT	~ WT + APH + CREA
V/F	~ HT + CREA + HCT	~ HT	~ HT + HCT

Table 23. Covariate selection by regression methods and NONMEM.

Final population PK model of Tac in Asian adult and paediatric liver transplant patients and its parameters estimated using first-order (FO) and FOCE with interaction are shown in Tables 24 and 25, respectively. From the results, it can be seen that the parameter estimates derived from the two methods of estimation were similar. Strong trends were absent in the basic goodness-of-fit plots for the final population model as shown in Figure 33, and indicated a reasonable model fit to the observed data. WRES vs PRED for the final population PK model is shown in Figure 34. The etas (random parameters) associated with the final model are normally distributed with mean of zero (Figure 35). Figure 36 illustrates the whole blood concentration-time profiles for two typical subjects together with the predicted concentrations arising from the population and the 'posthoc' individual parameter estimates.

The typical absorption rate constant, k_a, was 2.08 hr^{-1}, which corresponds to a population mean first-order absorption half-life, t_{abs}, of 0.33 hr (derived from the expression, $t_{abs} = 0.693 \div k_a$). The population mean first-order elimination constant, k, was found to be 0.065 hr^{-1} (derived from the expression, $k = \frac{CL/F}{V/F}$) and the corresponding population mean $t_{½}$ is 10.7 hr (derived from the expression, $t_{½} = 0.693 \div k$). The population mean time to maximum blood concentration (t_{max}) was 1.72 hr, calculated from $t_{max} = \frac{1}{k_a - k} \times \ln(\frac{k_a}{k})$.

Table 24. Final population PK model of Tac in Asian adult and paediatric liver transplant patients and its parameters.

Parameter	Symbol	Units	Estimated value	Precision of estimation Std. error	CV (%)
CL/F	Θ_{CL}	L/hr	14.1	1.93	13.7
V/F	Θ_{V}	L	217	36.6	16.9
k_a	Θ_{k_a}	hr^{-1}	2.08	0.292	14.0
Factor for WT on CL/F	Θ_{CL}^{WT}	$L.hr^{-1}kg^{-1}$	0.237	0.0517	21.8
Factor for APH ≥ 200 U/L on CL/F	Θ_{CL}^{APH}	L/hr	-2.93	1.17	39.9
Factor for CREA on CL/F	Θ_{CL}^{CREA}	$L^2.hr^{-1}.\mu mol^{-1}$	-0.0801	0.0357	44.6
Factor for HCT on V/F	Θ_{V}^{HCT}	L^2/L	-7.83	3.59	45.8
Factor for HT on V/F	Θ_{V}^{HT}	L/m	179	37.3	20.8
Interpatient variance of CL/F_j about CL/F_{TV}	$\omega^2_{CL/F}$		0.432	0.159	36.8
Interpatient variance of V/F_j about V/F_{TV}	$\omega^2_{V/F}$		0.407	0.195	47.9
CV of CL/F_j about CL/F_{TV}	$CV_{CL/F}$	%	65.7		
CV of V/F_j about V/F_{TV}	$CV_{V/F}$	%	63.8		
Intrapatient variance of C_{ij} about $C_{pred,ij}$	σ^2		0.121	0.0321	26.5
CV of C_{ij} about $C_{pred,ij}$	σ	%	34.8		

Structural models:

$$TV(CL/F) = \Theta_{CL} + \Theta_{CL}^{WT} * (WT - 55) + \Theta_{CL}^{APH} * Y + \Theta_{CL}^{CREA} * (CREA\text{-}60)$$

$$TV(V/F) = \Theta_{V} + \Theta_{V}^{HCT} * (HCT - 31.1) + \Theta_{V}^{HT} * (HT\text{-}1.61)$$

$$TVK_a = \Theta_{k_a}$$

Random effects models:

$$CL/F_j = TV(CL/F) * \exp(\eta_{j,CL})$$

$$V/F_j = TV(V/F) * \exp(\eta_{j,V})$$

$$C_{ij} = C_{pred,ij} * (1 + \epsilon_{ij})$$

where, WT = weight in kg; APH = alkaline phosphatase in U/L; CREA = creatinine in μmol/L; HCT = haematocrit in L/L; HT = height in m; TV(CL/F) = typical population value for CL/F; TV(V/F) = typical population value for V/F; and the value of Y is 0 for APH < 200 U/L. The Y value for APH ≥ 200 U/L is 1.

Table 25. Final population PK model of Tac in Asian liver transplant patients and its parameters estimated using FOCE.

Parameter	Symbol	Units	Estimated value	Precision of estimation Std. error	CV (%)
CL/F	Θ_{CL}	L/hr	19.5	3.2	16.4
V/F	Θ_{V}	L	267	48.4	18.1
k_a	Θ_{k_a}	hr^{-1}	1.67	0.311	18.6
Factor for WT on CL/F	Θ_{CL}^{WT}	$L.hr^{-1}kg^{-1}$	0.331	0.0914	27.6
Factor for APH ≥ 200 U/L on CL/F	Θ_{CL}^{APH}	L/hr	-3.91	1.28	32.7
Factor for CREA on CL/F	Θ_{CL}^{CREA}	$L^2.hr^{-1}.\mu mol^{-1}$	-0.0549	0.0354	64.5
Factor for HCT on V/F	Θ_{V}^{HCT}	L^2/L	-9.99	3.53	35.3
Factor for HT on V/F	Θ_{V}^{HT}	L/m	241	52	21.6
Interpatient variance of CL/F_j about CL/F_{TV}	$\omega^2_{CL/F}$		0.361	0.0614	17.0
Interpatient variance of V/F_j about V/F_{TV}	$\omega^2_{V/F}$		0.418	0.131	31.3
CV of CL/F_j about CL/F_{TV}	$CV_{CL/F}$	%	60.1		
CV of V/F_j about V/F_{TV}	$CV_{V/F}$	%	64.7		
Intrapatient variance of C_{ij} about $C_{pred,\,ij}$	σ^2		0.145	0.0304	21.0
CV of C_{ij} about $C_{pred,\,ij}$	σ	%	38.1		

Structural models:

$$TV(CL/F) = \Theta_{CL} + \Theta_{CL}^{WT} * (WT - 55) + \Theta_{CL}^{APH} * Y + \Theta_{CL}^{CREA} * (CREA\text{-}60)$$

$$TV(V/F) = \Theta_{V} + \Theta_{V}^{HCT} * (HCT - 31.1) + \Theta_{V}^{HT} * (HT\text{-}1.61)$$

$$TVK_a = \Theta_{k_a}$$

Random effects models:

$$CL/F_j = TV(CL/F) * \exp(\eta_{j,CL})$$

$$V/F_j = TV(V/F) * \exp(\eta_{j,V})$$

$$C_{ij} = C_{pred,\,ij} * (1 + \epsilon_{ij})$$

where, WT = weight in kg; APH = alkaline phosphatase in U/L; CREA = creatinine in μmol/L; HCT = haematocrit in L/L; HT = height in m; TV(CL/F) = typical population value for CL/F; TV(V/F) = typical population value for V/F; and the value of Y is 0 for APH < 200 U/L. The Y value for APH ≥ 200 U/L is 1.

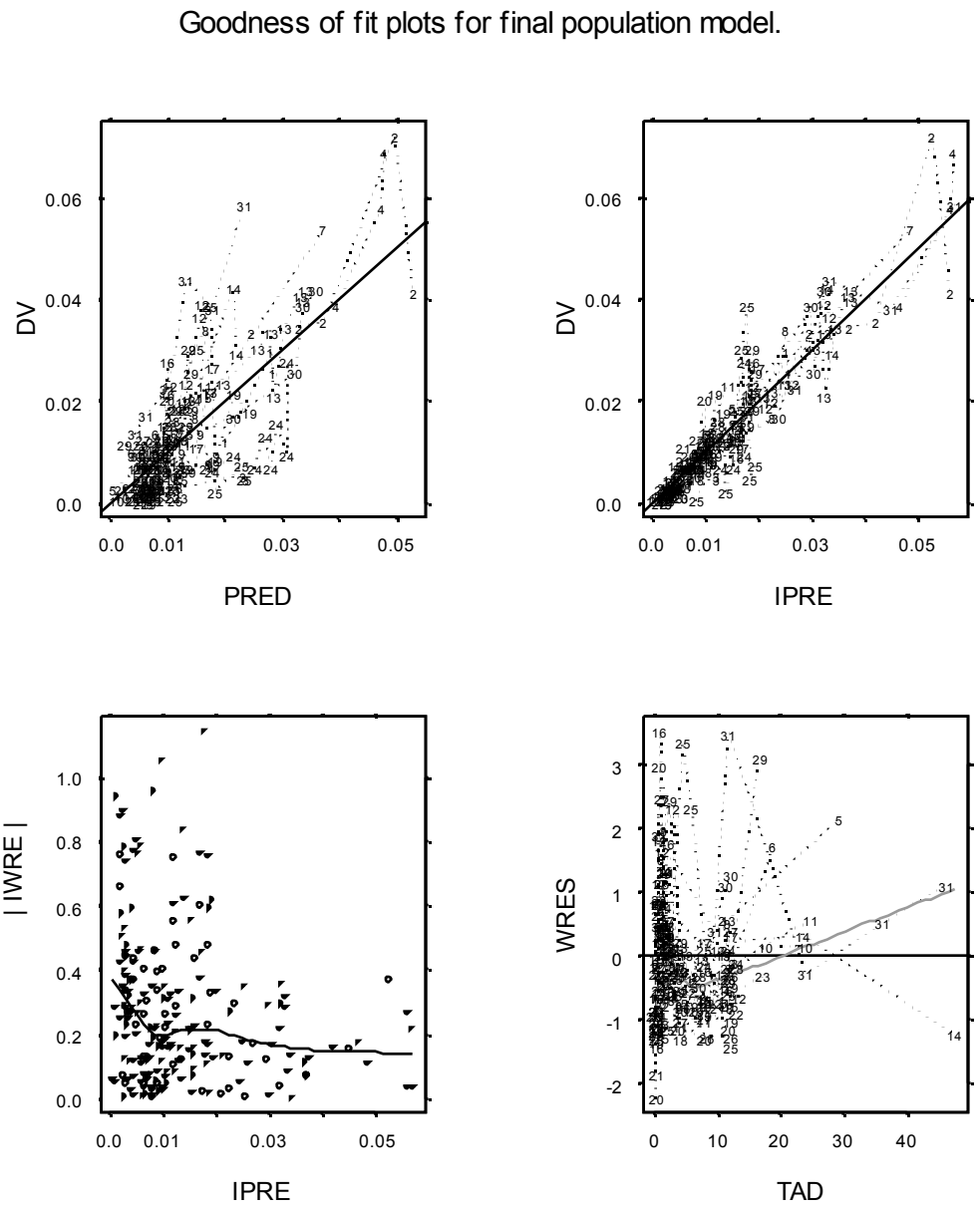


Figure 33. Basic goodness of fit plots for the final population model. The upper left panel shows the predictions for the typical individual in the population (PRED) vs the dependent variable (DV), i.e. the observed concentrations. The upper right panel is a plot of the individual predictions (IPRE) vs the dependent variable. The lower left is a plot of the absolute values of the individual weighted residuals (IWRE) vs the individual predictions. The lower right panel is a plot of the weighted residuals (WRES) vs the independent variable (TIME AFTER DOSE, TAD). In all but the lower left panel, each individual data points are connected by a line and labeled with the ID number. In the uppermost two panels, the solid line is the line of identity. In the lower left panel, the solid line is a smooth.

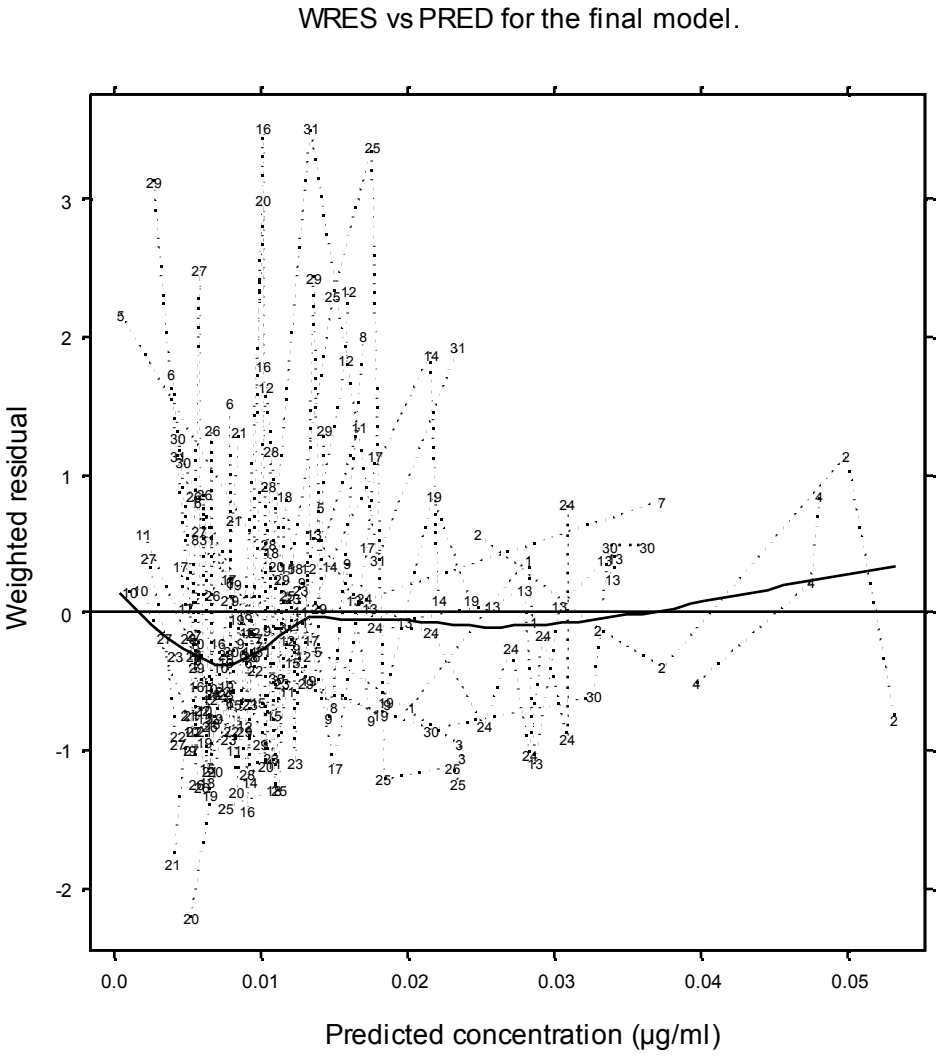
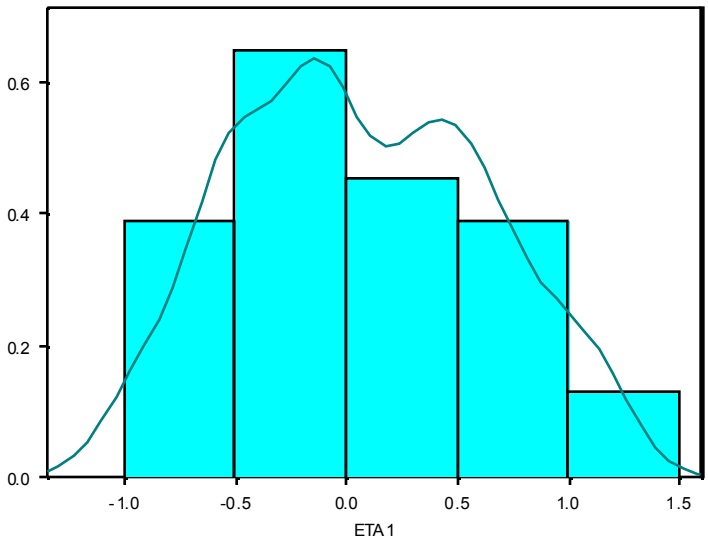


Figure 34. WRES vs PRED for the final population PK model. The solid line is a smooth.

(A)



(B)

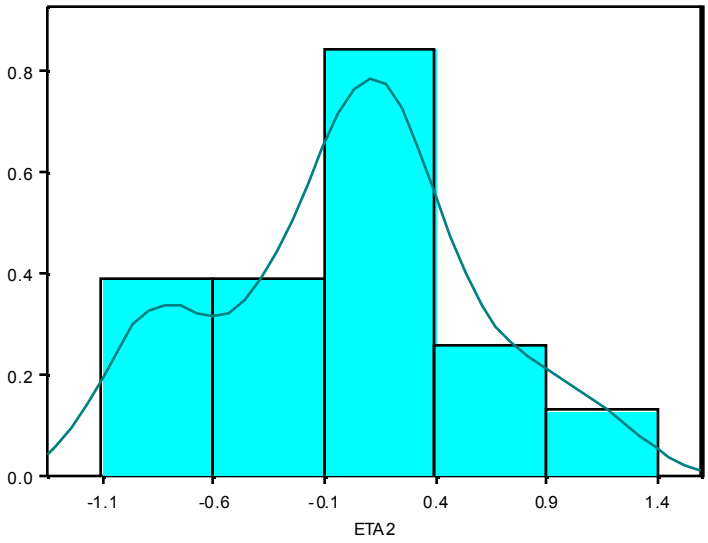
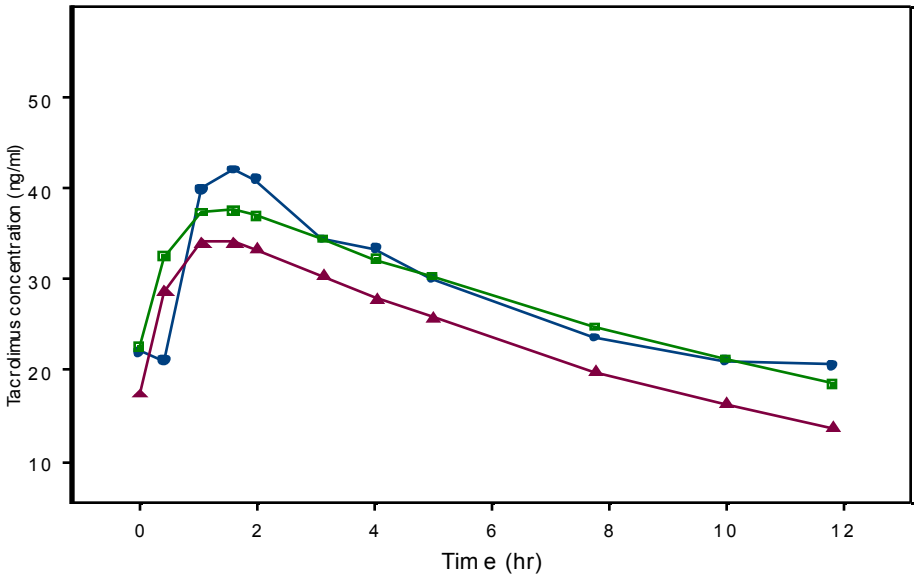


Figure 35. Histogram with density line plots showing the distribution of (A) ETA1; and (B) ETA2 of the final model.

(A)



(B)

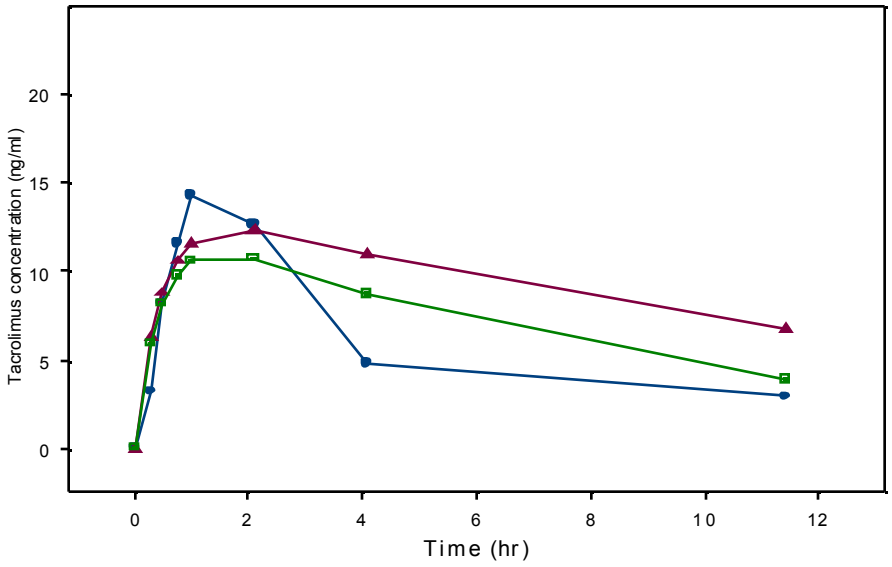


Figure 36. Whole blood Tac concentration-time profiles from (A) a 14 year old male paediatric patient; and (B) a 60 year old male adult patient, showing measured (●), population predicted (▲) and individual predicted (■) concentrations from the full population PK model for Tac.

CL/F for a representative patient, weighing 55 kg, APH < 200 U/L and CREA 60 μmol/L, was 14.1 L/hr. CL/F increased by 0.237 L/hr per kg WT; hence a 1-kg increase in body weight resulted in a 1.7 % increase in CL/F. CL/F decreased by 2.93 L/hr when APH ≥ 200 U/L; therefore APH ≥ 200 U/L was associated with a 20.8 % reduction in CL/F. CL/F decreased by 0.0801 L/hr per μmol/L increase in CREA; hence 1 μmol/L increase in CREA was associated with a 0.6 % reduction in CL/F.

V/F for a representative patient, of HT 1.61 m and HCT of 31.1 L/L, was 217 L. V/F decreased by 7.83 L per 1 L/L increase in HCT; hence a 1 L/L increase in HCT was associated with a 3.6 % reduction in V/F. V/F increased by 179 L per m in HT; therefore every 1 m increase in HT was associated with a 82.5 % increase in V/F.

The population mean CL/F of 14.1 or 19.5 L/hr obtained in this study can be compared with that obtained in the first study involving the paediatric patients which has a mean population value of 7.41 L/hr. Thus, it can be seen that the CL/F estimated in the second study, has a value that is about twice that of the first study. Also, the population mean V/F of 217 or 267 L obtained in this study has a higher value than the value of 198 L obtained in the first study. Possible reasons for the difference in the values of these estimates include differences in the patient composition and the difference in the analytical methods used for quantification of tacrolimus in these two studies.

Case deletion diagnostics for the 30 single case-deleted reanalyses of the final model are given in Table 26. CVs for parameter estimates from each reanalysis were less

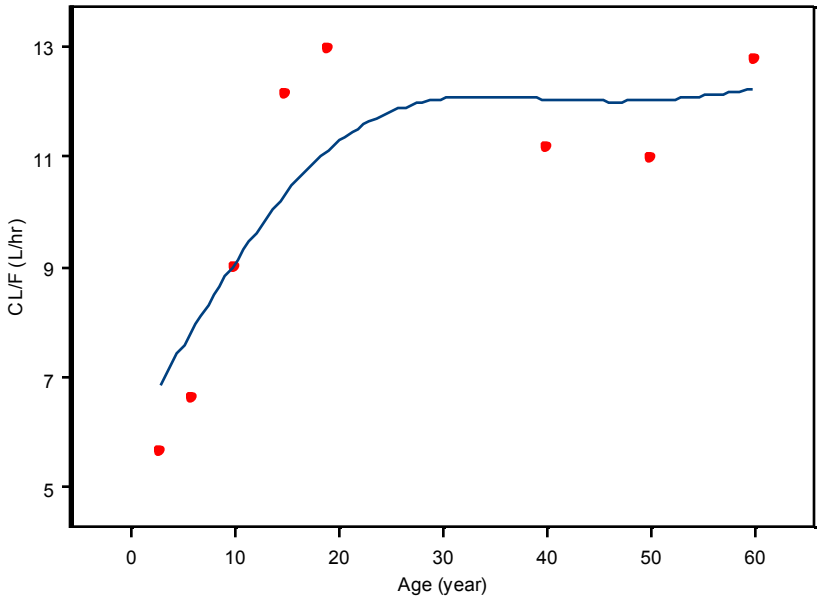
than 15 % for all parameters. Using the population PK model derived in this study, values of covariates WT and HT (Leung *et al.*, 1996; Epidemiology and Disease Control Department, 1998; Franklin, 1999), CREA (Jury, 1979) and HCT (McPherson *et al.*, 1978; Yip *et al.*, 1984) were obtained from the literature to be used for the calculation of CL/F and V/F for male patients of the ages 3 to 60 years old. These PK parameters were then used for the calculation of WT-normalized CL/F, V/F and doses needed to achieve a desired steady-state whole blood trough concentration of 10 ng/mL for male patients of the ages between 3 to 60 years old. Graphical plots of these results are shown in Figures 37, 38 and 39.

Table 26. Case deletion diagnostics for evaluation of final model estimates.

Parameter	Mean	CV(%)	Minimum[a]	Maximum[a]
Θ_{CL}	14.15	3.27	13.3	15.3
Θ_{V}	217.68	4.73	194	243
Θ_{k_a}	2.09	3.59	1.98	2.35
Θ_{CL}^{WT}	0.238	5.02	0.202	0.264
Θ_{V}^{HCT}	-7.93	13.1	-10.5	-4.9
Θ_{V}^{HT}	180.16	6.82	152	227
Θ_{CL}^{APH}	-2.95	11.7	-4.2	-2.19
Θ_{CL}^{CREA}	-0.0799	14.2	-0.103	-0.0367

[a]Minimum and maximum values of all 30 estimates (each from one case deletion re-run of final model).

(A)



(B)

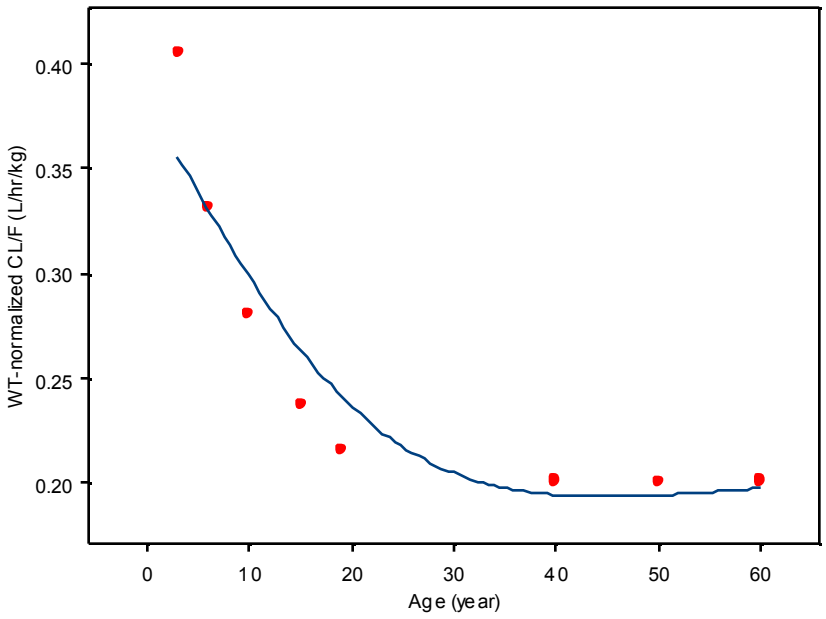
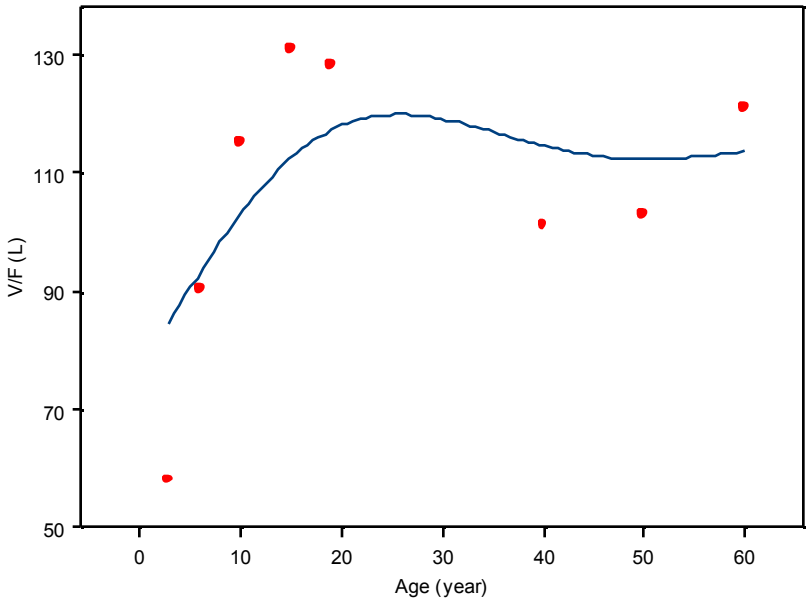


Figure 37. Relationships between (A) age and CL/F; and (B) age and WT-normalized CL/F. Solid line is a spline smooth.

(A)



(B)

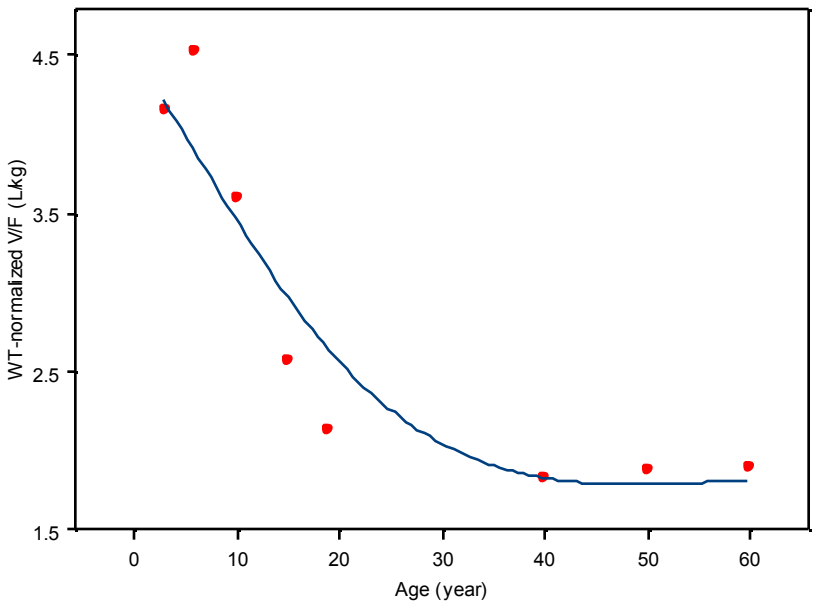


Figure 38. Relationships between (A) age and V/F; and (B) age and WT-normalized V/F. Solid line is a spline smooth.

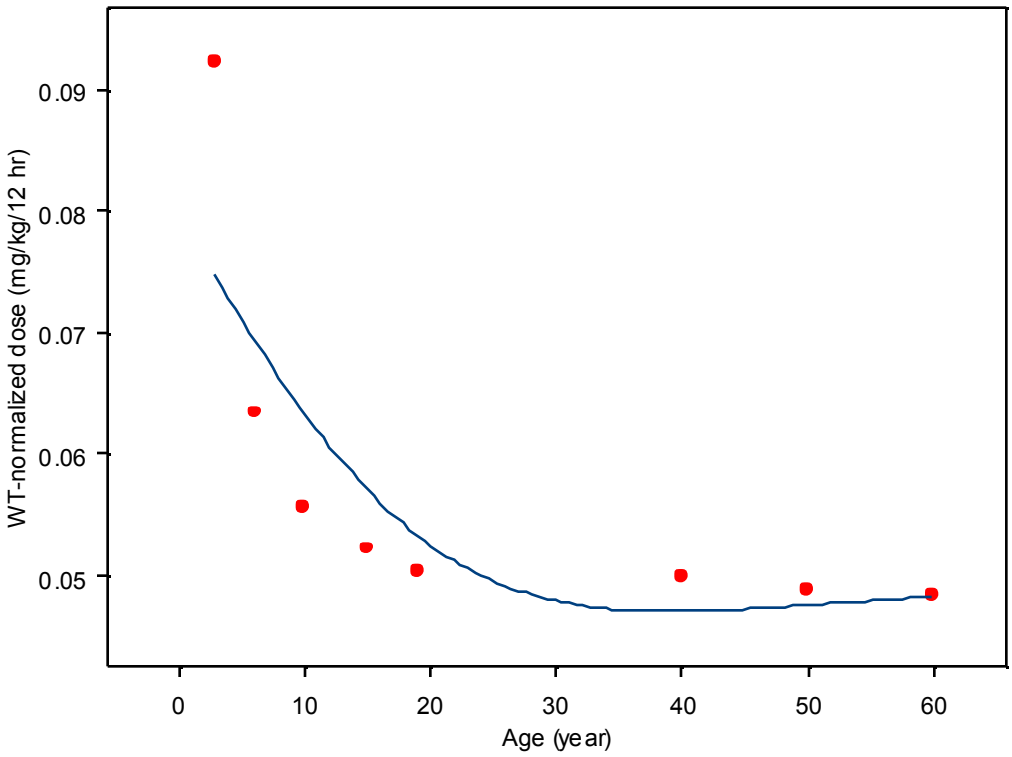


Figure 39. Relationship between age and WT-normalized dose needed to achieve a desired steady-state whole blood trough concentration of 10 ng/mL. Solid line is a spline smooth.

4.4. Discussion

Using population techniques, a detailed PK analysis of Tac was performed in Asian adult and paediatric liver transplant patients. Such techniques are able to analyze sparse and dense data sets, quantitatively assess the influence of patient covariates on drug disposition, and ultimately provide dosing regimen algorithms for optimal individualised dosing (Whiting *et al.*, 1986; Ludden, 1988).

In this study, we estimated Tac PK parameters in conjunction with common clinical covariates to build a model to predict individual Tac CL/F and V/F that may be used in the clinical setting to estimate dose requirements. The mean population k_a of 2.08 hr^{-1} determined in this study is about half compared to 4.48 hr^{-1}, a value which was reported in a previous study (Jusko *et al.*, 1995a). After oral administration, Tac was absorbed quickly with a mean t_{max} of 1.72 hr. This is similar to the mean t_{max} of 2.1 hr reported in a study in paediatric liver transplant patients (Wallemacq *et al.*, 1998).

We found a Tac PK population estimate of CL/F of 14.1 L/hr. For a representative paediatric patient (WT 18.4 kg, CREA 32.7 μmol/L, APH 166 U/L, HT 0.95 m and HCT 31.7 L/L) in this study, the calculated CL/F is 0.414 L/hr/kg or 7.62 L/hr. This is about twice the value of CL/F estimates of 0.21 L/hr/kg (estimated for a 15 kg patient when time after treatment initiation = 0, bilirubin = 0.6 mg/dL and normal ALT) reported following oral administration of Tac to paediatric liver transplant recipients being converted from cyclosporin to Tac (Sanchez *et al.*, 2001). This is also similar to the

population mean CL/F estimate of 5.75 L/hr obtained in paediatric cut-down liver transplant recipients (Staatz *et al.*, 2001). For a representative adult patient (WT 67.2 kg, CREA 98 μmol/L, APH 126 U/L, HT 1.66 m and HCT 34.7 L/L) in this study, the calculated CL/F is 0.208 L/hr/kg. This is similar to CL/F estimates of 0.25 L/hr/kg reported following oral administration of 5 mg Tac to caucasian subjects (Mancinelli *et al.*, 2001). As the two studies (Mancinelli *et al.*, 2001; Sanchez *et al.*, 2001) were performed on Caucasian patients, it appears that race does not have an effect on the CL/F of Tac because the population mean values of CL/F of these patients are comparable to that in Asian patients of similar age groups as determined in our study.

Part of the interindividual variability in the CL/F of Tac was explained by WT, elevated APH and serum CREA of the patient. The interindividual variability of CL/F in the basic model was 104.4 %; however, on including the patient's body WT, APH level and CREA, this variability is reduced to 65.7 % in the final population model. Thus, the CL/F of Tac is influenced by three factors: the body size, liver function and renal function.

The covariate submodel for CL/F has the greatest importance because of its possible role in dosage regimen design. GAM, TBM and NONMEM all showed that WT is the most important covariate influencing CL/F of Tac. Specifically, a 1-kg increase in body WT resulted in a 1.7 % increase in CL/F. Hence, at two extremes of weight, average 45 kg and 80 kg patients (with normal APH and CREA 60 μmol/L) would have CL/F of 11.7 L/hr and 20.0 L/hr, respectively. Hence, dose adjustment of Tac for WT appears

necessary. This finding is of particular clinical relevance because it indicates that dosing on a mg per kg basis would decrease the variability in concentration-time profiles of Tac into a narrower range for the paediatric and adult Asian liver transplant patients. Therefore, the current practice of dosing of oral Tac by WT is justified in liver transplant patients.

In this study it has been found that a rise in APH ≥ 200 U/L was independently associated with a reduction in Tac CL/F of 2.93 L/hr. APH, a variable which reflects hepatocellular alterations and biliary excretion, was found to have an effect on CL/F, but only when it is incorporated into the model as a discrete variable with a cut-off value of 200 U/L. Tac is primarily metabolized in the liver and intestinal mucosa by the cytochrome P4503A4 enzyme, and eliminated through biliary excretion. Therefore, patients with poor liver function will have reduced Tac CL/F as compared with patients with normal liver function. This is consistent with the results of previous studies in adult liver transplant patients, which reported that hepatic dysfunction can decrease Tac CL and increase $t_{½}$ (Jain *et al.*, 1990; Jain *et al.*, 1993).

Serum CREA has been found to influence the CL/F of Tac in this study. Specifically, a 1-μmol/L increase in CREA was associated with a 0.6 % reduction in CL/F of Tac. Hence, at two extremes of CREA, patients with 60 μmol/L and 120 μmol/L (with normal APH and WT 55 kg) would have CL/F of 14.1 L/hr and 9.3 L/hr, respectively. Hence, dose adjustment of Tac for CREA appears unnecessary. Renal CL of Tac is less than 1 % of total body CL (Venkataramanan *et al.*, 1991). Therefore the

influence of CREA on Tac CL/F is unlikely to be a reduction in renal CL. Results from previous studies of the influence on renal function on the CL of Tac have been contradictory. Some studies reported no significant correlation between serum CREA concentration and CL of Tac (Gruber *et al.*, 1994; Staatz *et al.*, 2003) while other studies have found such a correlation. The results of a study showed that a rise in CREA ≥ 2 mg/dL was independently associated with a 40 % reduction in Tac CL in hematopoietic cell transplant patients (Jacobson *et al.*, 2001). The authors hypothesized that a high CREA may serve as a surrogate of altered renal blood flow resulting from subclinical hepatic injury. A study reported that the CL of recipients with renal dysfunction, which was defined as serum CREA over 1 mg/dL, was 80.9 % of that in recipients with serum CREA below this level (Fukatsu *et al.*, 2001). The findings were hypothesized to be due to alterations in drug metabolism related to the hepatorenal syndrome. The conflicting results of the influence of CREA on CL/F of Tac in the different studies may be due to the different ranges of CREA encountered in the patients of the different studies.

A study in rats showed that the F of Tac was increased by about 35 % in cisplatin-induced acute renal failure rats (Okabe *et al.*, 2000). This was due to two mechanisms: impaired hepatic metabolism of Tac during renal dysfunction, and increased absorption rate of Tac in the intestine of rats with renal dysfunction, which leads to a partial saturation of hepatic extraction (Okabe *et al.*, 2002). Therefore, although renal failure is commonly thought to affect only the renal elimination of drugs, it may have different influences on the PK of a drug (Gibson, 1986). Thus, impaired hepatic metabolism and

increased F are possible mechanisms explaining the effect of renal dysfunction on CL/F of Tac in humans as observed in this study.

The following example illustrates how the covariate sub-model for CL/F of Tac may be used in the clinical setting: A 60 kg adult with an APH of 160 U/L and CREA of 80 μmol/L will have an estimated CL/F of: CL/F (L/hr) = population CL/F estimate + 0.237∗(WT-55) – 2.93 – 0.0801∗(CREA-60) = 14.1 + 1.185 – 2.93 – 1.602 = 10.8.

The population estimate of V/F of 217 L is reasonable for a highly lipophilic compound like Tac. For a representative paediatric patient (WT 18.4 kg, CREA 32.7 μmol/L, APH 166 U/L, HT 0.95 m and HCT 31.7 L/L) in this study, the calculated V/F is 94.2 L (5.12 L/kg). This is similar to V/F of 9.0 L/kg reported following oral administration of Tac to paediatric liver transplant recipients (Wallemacq *et al.*, 1998). For a representative adult patient (WT 67.2 kg, CREA 98 μmol/L, APH 126 U/L, HT 1.66 m and HCT 34.7 L/L) in this study, the calculated V/F is 198 L (2.94 L/kg). This is about half of the V/F estimate of 399 L reported following oral administration of Tac to adult liver transplant recipients (Staatz *et al.*, 2003). As the two studies (Wallemacq *et al.*, 1998; Staatz *et al.*, 2003) were performed in Caucasian patients, it appears that race does not have an effect on the V/F of Tac because the population mean values of V/F of these patients are comparable to that in Asian patients of similar age groups as determined in our study.

Part of the interindividual variability in the V/F of Tac was explained by HT and HCT of the patient. The interindividual variability of V/F in the basic model was 113.1 %; however, on including the patient's HT and HCT, this variability is reduced to 63.8 % in the final population model. Thus, the V/F of Tac is affected by two factors: the body size and HCT. HT has been found to be a better indicator of body size and hence drug distributional space than WT, AGE and BSA in this group of patients. Our previous work of the population PK of Tac in Asian paediatric liver transplant patients showed that BSA correlates with the V of Tac. There was a negative association of HCT with V/F of Tac, which is a previously unreported finding. It supports our previous hypothesis made in the previous study of this thesis, regarding the effect of haematocrit on the V of Tac, based on the phenomenon that Tac is highly bound to red blood cells (Nagase *et al.*, 1994). Therefore, an increase in HCT would decrease the partitioning of Tac into fat, thereby decreasing its V.

The following example illustrates how the covariate sub-model for V/F of Tac may be used in the clinical setting: A 1.65 m adult with a haematocrit of 35 L/L will have an estimated V/F of: V/F (L) = population V/F estimate – 7.83∗(HCT-31.1) + 179∗(HT-1.61) = 217 – 30.5 + 7.16 = 193.7.

Once the approximate Tac CL/F and V/F of an individual is known, an estimate of the dose needed to achieve a desired steady-state whole blood trough concentration is possible using the following equation:

$$\text{Dose} = \frac{(C_{SS,\min} \times V / F \times \left[1 - e^{-k_e \tau}\right])}{e^{-k_e \tau}}$$

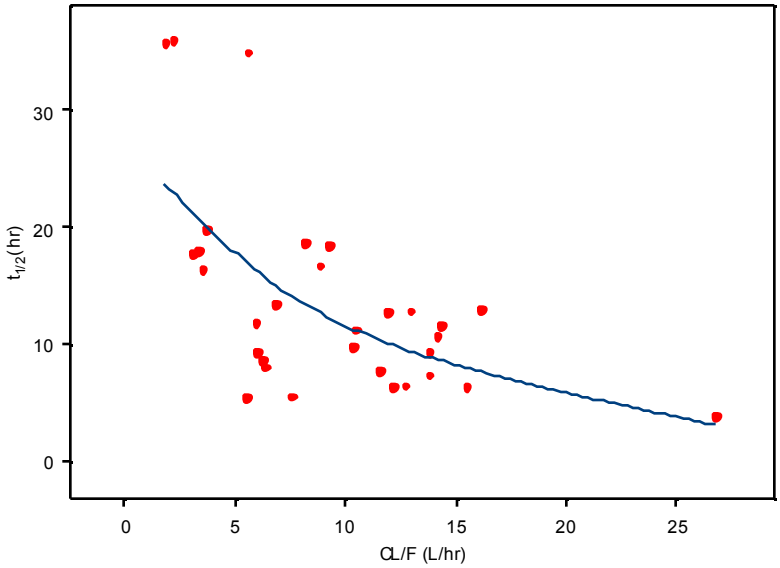
where $C_{SS,\min}$ is the desired steady-state whole blood trough concentration, k is elimination rate constant and τ is the dosing interval. The mean population PK parameters provided by the population model derived in this study may be useful in TDM for the estimation of an individual's PK parameters by using Bayesian methods, which require prior information about population kinetic parameters of the population.

Among other PK parameters estimated in this study, the $t_{½}$ is of importance for the clinical use of any drug and it should be considered. Figure 40 shows that the $t_{½}$ of Tac is inversely proportional to CL/F and is not influenced by V/F. Thus, any factor reducing CL/F (e.g. reduced WT and elevated APH or CREA) will prolong the $t_{½}$ of Tac. The mean population $t_{½}$ of 10.7 hr derived in this study is similar to the mean $t_{½}$ of 12.1 hr reported in one study (Jusko *et al.*, 1995a).

The current population model for Tac has appreciably reduced interpatient variabilities in CL/F and V/F. It can be further seen that the patient covariates identified in this model have a large impact on dosage regimen design (Figure 41) in which wide range of doses were required to achieve a target steady-state trough concentration of 10 ng/mL.

The population PK analysis using NONMEM also estimated interindividual and residual variabilities in addition to mean PK parameters. Reasonably large interindividual

(A)



(B)

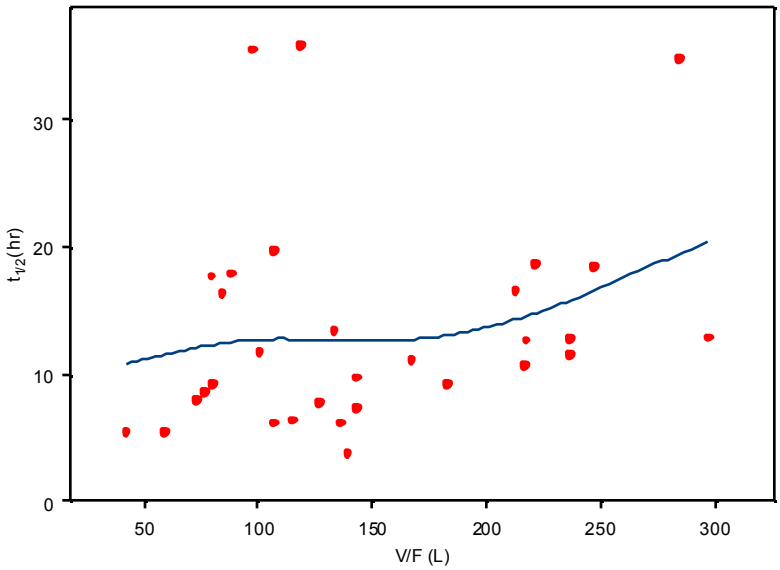
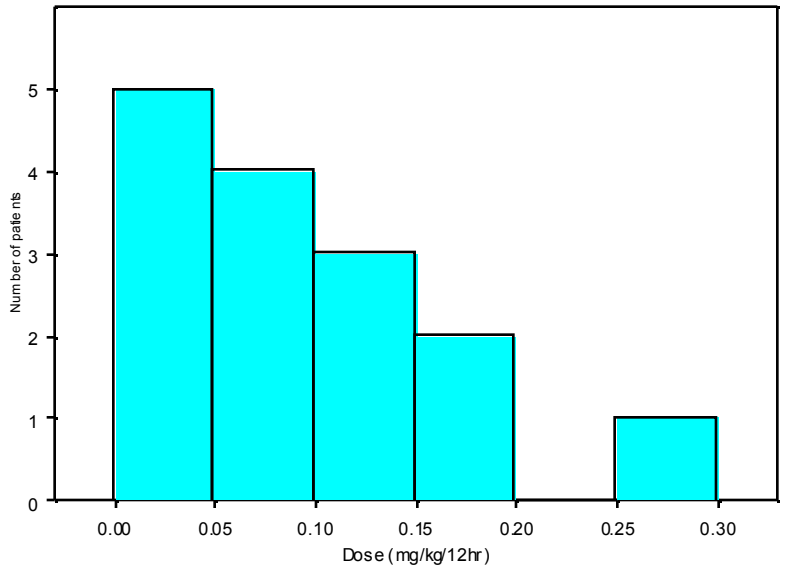


Figure 40. Individual estimates obtained for the $t_{½}$ in all patients included in the dataset as plotted against (A) Tac CL/F; and (B) Tac V/F. The solid line in each plot is a spline smooth.

(A)



(B)

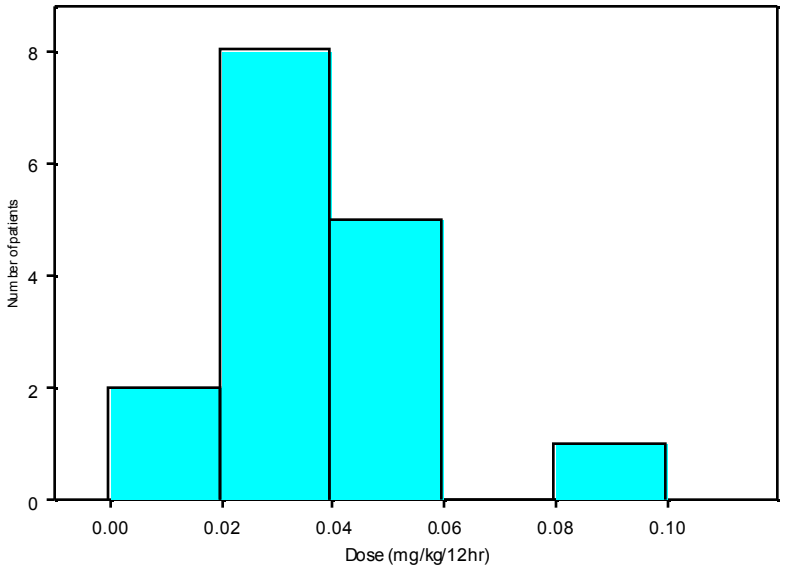


Figure 41. Model-predicted Tac doses (mg/kg/12hr) required to reach a target steady-state trough concentration of 10 ng/ml in (A) 15 paediatric patients; and (B) 16 adult patients.

variabilities of 65.7 % and 63.8 % were estimated for CL/F and V/F, respectively. This may be because each of these parameters (i.e. CL/F and V/F) is a composite of two parameters. Thus the interindividual variabilities estimated are the result of interindividual variabilities in CL or V in addition to F. Interindividual variabilities in F of Tac have been reported to be 63.0 % (Fukatsu *et al.*, 2001) and 21.0 % (Yasuhara *et al.*, 1995) in Japanese adult and paediatric liver transplant patients, respectively. Reasonably moderate residual variability of 34.8 % was estimated.

It can be seen that the weight-normalized dose decreases steadily from a value of 0.092 mg/kg/12 hr at an age of 3 years to a value of 0.05 mg/kg/12 hr at an age of 19 years and remains constant until an age of 60 years. This shows that paediatric patients require about twice as much of the weight-normalized dose compared to adult patients to maintain the same steady-state whole blood trough concentrations, an observation which is consistent with that reported in the literature (McDiarmid *et al.*, 1993). It also shows that the population PK model derived in this study is able to offer an accurate prediction of a clinically observed phenomenon.

Information derived from this population model may subsequently be used for dosage individualization through Bayesian forecasting (Thomson and Whiting, 1992). Bayesian estimation uses prior parameter probability distributions, together with the patient's measured serum concentration(s), to arrive at values for the PK parameters that are most likely to represent the patient's true values when data are sparse. PK parameters for each patient become individualized and the influence of the population parameters

decreases(Fernandez de Gatta *et al.*, 1996). Optimally, these techniques also inform the clinician of the next appropriate dose to administer in order to maintain or reach a desired concentration. The number of blood collections needed and the time to reach the required drug concentrations can be reduced. The advantage of Bayesian modeling is that the presence of the *a priori* population estimates restricts the newly estimated parameter values so that they are less likely to give odd combinations that simply happen to fit the data. Therefore, ultimately, a population model for Tac in conjunction with Bayesian forecasting may lead to drug-dosing algorithms that can individualize a patient's dose, thereby increasing the efficacy and decreasing the toxicity of Tac.

CONCLUSIONS

5. Conclusions

The clinical PK of Tac was investigated in Asian paediatric and adult liver transplant patients using the population approach, which is the focus of this research work. Population methods are particularly appropriate in these patients because they allow the modeling of PK responses in a relatively large group of patients with sparse data obtained during routine TDM. Moreover, population models would offer the possibility of better dosage individualization, both '*a priori*' and '*a posteriori*', using Bayesian forecasting. If a patient has not yet received a drug, Bayesian forecasting can provide an initial dosage recommendation based on population values. However, if drug concentrations are available through TDM, they can be used as feedback and the Bayesian program will provide new recommendations based on both population and patient-specific values. As more drug levels are used as feedback, the forecasted doses will shift towards patient-specific values and less population-based.

For the second study in this thesis, it is the first report of Tac population PK study which included both adult and paediatric Asian liver transplant patients. PK parameters derived in the two studies were comparable to those reported in earlier studies on subjects of similar ages (Wallemacq *et al.*, 1998; Mancinelli *et al.*, 2001; Sanchez *et al.*, 2001; Staatz *et al.*, 2003). Several patient characteristics, which have significant influence on the PK parameters of Tac have been identified and their relationships to the PK parameters elucidated. These patient covariates have explained a large portion of the interindividual variabilities in the PK parameters of Tac upon inclusion into the

population models. Future studies are necessary involving more covariates so as to explain a larger portion of the interindividual variabilities in the PK parameters of Tac.

The results of this study show that the PK parameters of Tac are influenced by the developmental characteristics and clinical conditions of the patient. As liver and renal functions of the patient are factors affecting the PK of Tac, these should be routinely monitored. This is especially critical in the early post-transplant period in which pathophysiological changes can occur rapidly in the transplant patients. Tac concentrations falling outside the therapeutic range may result in toxicity or therapeutic failures; therefore, rapid adjustment of doses is imperative.

The results of the study support the current clinical practice of dosing oral Tac by the patient‘s body WT. Given these findings, the mean population PK parameters can be estimated and then applied to compute dosages of Tac in Asian adult and paediatric liver transplant patients. However, these should be applied with caution because of the unexplained variability in the PK parameters. Therefore, a close monitoring of Tac whole blood concentrations, especially during the first month of transplantation, is recommended. Furthermore, although the patients included in the two studies are representative of the general Asian liver transplant population, it is insufficient for establishing a definitive model for dose recommendation for these patients and other patients of non-Asian origins. Thus, it represents the limitation of the work in this thesis. This is because there are only a limited number of liver transplants performed locally. For example, there were only 46 cases of liver transplants performed in Singapore between

1995 and 1999 (Wai *et al.*, 2000). Thus future studies involving larger numbers of patients will be necessary to confirm the present findings.

The patient groups included in the two studies are representative of "real-life" Asian liver transplant recipients. The dosages of Tac given to different subjects (and also during the course of each patient's therapy) varied considerably. The data collected in the two studies characterized that which is normally available for transplant patients. Thus findings from this thesis such as the mean population pharmacokinetic estimates should be applicable to the larger Asian liver transplant community.

Finally, using the population models derived from the two studies in this thesis, in conjunction with Bayesian forecasting, a truly individualized immunosuppressant therapy can be developed and applied for Asian liver transplant patients.

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